

ENZYMATIC OXIDATION OF AROMATIC SUBSTRATES VIA TOLUENE  
DIOXYGENASE AND CATECHOL DEHYDROGENASE: APPLICATION TO  
TOTAL SYNTHESIS OF COMBRETASTATINS A-1 AND B-1

By

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Dedicated to my grandmother, Nguyen Thi Sau

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## LIST OF ABBREVIATIONS

|                |                                                             |
|----------------|-------------------------------------------------------------|
| AIBN           | azoisobutyronitrile                                         |
| aq.            | aqueous                                                     |
| BDO            | benzene dioxygenase                                         |
| Bn             | benzyl                                                      |
| borax          | sodium borate-decahydrate                                   |
| BPDO           | biphenyl dioxygenase                                        |
| BVMO           | Baeyer-Villiger Monooxygenase                               |
| Bz             | benzoyl                                                     |
| BZDO           | benzoate dioxygenase                                        |
| CHMO           | cyclohexanone monooxygenase                                 |
| CSP-HPLC       | chiral stationary phase-high pressure liquid chromatography |
| DEAD           | diethylazodicarboxylate                                     |
| DIBAL          | diisobutyl aluminium hydride                                |
| DMAP           | <i>N,N'</i> -dimethyl-4-amino pyridine                      |
| DMDP           | 2,5-dideoxy-2,5-diimino-D-mannitol                          |
| DMF            | dimethylformamide                                           |
| DMP            | 2,2-dimethoxypropane                                        |
| DMS            | dimethylsulfide                                             |
| DMSO           | dimethylsulfoxide                                           |
| <i>E. coli</i> | <i>Escherichia coli</i>                                     |

|                        |                                                                                 |
|------------------------|---------------------------------------------------------------------------------|
| ee                     | enantiomeric excess                                                             |
| EtOAc                  | ethyl acetate                                                                   |
| Eu(hfc) <sub>3</sub> ) | tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]<br>europium (III),   |
| HPLC                   | high-performance liquid chromatography                                          |
| HRMS                   | high resolution mass spectroscopy                                               |
| IPTG                   | iso-propyl- $\beta$ -D-thiogalacto-pyranoside                                   |
| KOH                    | potassium hydroxide                                                             |
| LAH                    | lithiumaluminum hydride                                                         |
| LDA                    | lithium diisopropylamide                                                        |
| L-DOPA                 | 3,4-dihydroxy-phenyl alanine                                                    |
| <i>m</i> -CPBA         | <i>meta</i> -chloroperbenzoic acid                                              |
| MOMCl                  | methoxymethyl chloride                                                          |
| MPB                    | 2-(1-methoxyethyl)benzeneboronic acid                                           |
| MPLC                   | medium-pressure liquid chromatography                                           |
| MsCl                   | methylsulfonyl chloride                                                         |
| MPA                    | R- <i>or</i> S- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenylacetic acid |
| NDO                    | naphthalene dioxygenase                                                         |
| NMNO                   | <i>N</i> -methylmorpholine- <i>N</i> -oxide                                     |
| NOEDS                  | nuclear overhauser effect difference spectroscopy                               |
| OD                     | optical density                                                                 |
| P-450                  | cytochrome P-450                                                                |
| PAD                    | potassium azodicarboxylate                                                      |

|                  |                                               |
|------------------|-----------------------------------------------|
| PAHs             | polycyclic aromatic hydrocarbons              |
| PCBs             | polychlorinated biphenyls                     |
| PCC              | pyridinium chlorochromate                     |
| <i>P. putida</i> | <i>Pseudomonas putida</i>                     |
| pyr.             | pyridine                                      |
| TBDPSCI          | chloro- <i>tert</i> -butyldiphenylsilane      |
| TBDMSCI          | <i>t</i> -butyldimethylsilyl chloride         |
| TBSCl            | chloro- <i>tert</i> -butyldimethylsilane      |
| TBTH             | tributyl-tin hydride                          |
| TDDH             | toluene <i>cis</i> -dihydrodiol dehydrogenase |
| TDII             | <i>N,N'</i> -thiocarbonyldiimidazole          |
| TDO              | toluene dioxygenase                           |
| TFA              | trifluoroacetic acid                          |
| THF              | tetrahydrofuran                               |
| TLC              | thin-layer chromatography                     |
| TsCl             | <i>para</i> -toluenesulfonyl chloride         |
| <i>p</i> -TsOH   | <i>para</i> -toluenesulfonic acid             |
| UV               | ultraviolet                                   |
| VIS              | visible                                       |

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Abstract of Dissertation Presented to the Graduate School  
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Chair: Tomas Hudlicky

Major Department: Chemistry

Toluene dioxygenase (TDO)-carrying microorganisms, the *Pseudomonas putida* F39/D and the genetically engineered *Escherichia coli* JM109 (pDTG601), were used to investigate the enzyme's kinetic resolution power in resolving aromatic substrate with remote chiral center. In the biotransformation of 1-phenyl-ethanol with *P. putida* F39/D, the S-enantiomer was consumed at faster rate than its R-counter part. However, when the same substrate was oxidized using *E. coli* JM109 (pDTG601), both enantiomers were oxidized indiscriminately at the same rate. Similar substrates with larger substituents at the stereogenic center were also oxidized without any preference for either enantiomer by JM109 (pDTG601). Biotransformation of cyclopropylbenzene was carried out to investigate whether or not the biotransformation process goes through a radical intermediate. New metabolites obtain in these studies were isolated, characterized, and their absolute stereochemistry were established through conventional chemical manipulations.

A series of substituted catechols were made using *E. coli* JM109 (pDTG602), which carries the toluene cis-dihydrodiol dehydrogenase (TDDH), as the source of biocatalyst. 4-Bromoanisole catechol obtained through biotransformation of corresponding arene, 4-bromoanisole, with JM109 (pDTG602) was used in the total syntheses of Combretastatins A-1 and B-1. (1S, 2S)-3-bromocyclohexa-3,5-diene-1,2-diol was also used as chiral synthon for synthesis of polyhydroxylated chiral polymer.

## CHAPTER I INTRODUCTION

Aromatic compounds are ubiquitous in the environment and originate from both natural and anthropogenic sources. The latter has become an issue of public concern for human health and environment, as more and more of these compounds find their ways into the ecosystem through misuse, accidental spillage, and improper disposal.<sup>1</sup> Although many aromatic hydrocarbons are biodegradable by existing microorganisms,<sup>2</sup> there are many man-made xenobiotics, such as polychlorinated biphenyls (PCBs) that are extremely stable and some are non-biodegradable.<sup>3-5</sup> As a result, bioaccumulation of these recalcitrant chemicals has occurred and has given rise to a whole host of biological problems including mortality, edema, hepatotoxicity, teratogenicity, immunotoxicity, reproductive toxicity, and promotion of cancer, affecting organisms at all levels.<sup>6,7</sup>

The toxic effect of aromatic hydrocarbons on living systems brought about awareness of the need for prevention and cleanup of already polluted areas. In this effort, bioremediation technologies have recently emerged as economical and practical viable alternatives to conventional physicochemical methods. Because of microorganisms' ability to adapt quickly to a changing environment, a very diverse collection of microorganisms now can be found in many kinds of extreme environments. These microorganisms are capable of metabolizing a variety of environmental contaminants, ranging from heavy metals to recalcitrant aromatic hydrocarbons. While a detailed review on bioremediation technology is beyond the scope of this discussion, it is evident

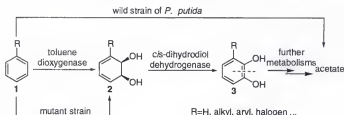
that microorganisms possess great potential that remains to be exploited for scientific endeavors.

In recent years, microbes have been recognized as indispensable biocatalysts for manufacturing enantiomerically pure materials in the chemical and pharmaceutical industries. Biocatalysts have been used to simplify and enhance the production process of complex chemicals and drug intermediates. They can add stereospecificity to the process, eliminating the need for complicated separation and purification steps.

In the field of organic synthesis, the application of enzymes has led to significant developments in stereoselective approaches to well-known reactions, such as aldol-condensations,<sup>8-14</sup> ketone-reductions,<sup>15,16</sup> Baeyer-Villiger oxidations,<sup>17,18</sup> and acyloin condensation.<sup>19-23</sup> Although the introduction of chirality is one of the main aims, the use of microbial enzymes as catalysts for the production of chemically useful compounds such as alcohols, acids, and amides has been the focus of attention for many years. In most cases, enzymes have been used for reactions difficult to achieve by purely chemical methods, e.g., regioselective functionalization of a non-activated carbon center or regioselective conversion of one out of several similar functional groups in a molecule. These tasks are facilitated by the mild conditions under which enzymatic reactions take place.

Recognizing the great potential of microorganisms as tools in synthesis, the last two decades have witnessed a steady growth of activity within the field.<sup>24-28</sup> Research groups from across the globe continue to explore for useful applications of microbes in their synthetic designs.

The Hudlicky research program is heavily involved in using the toluene dioxygenase-carrying microorganism, *Pseudomonas putida* F39D, as the source of biocatalyst for generation of chiral synthons for application toward numerous synthetic targets. The *P. putida* F39D was discovered by Gibson and colleagues in the late 1960s, and is noteworthy for its ability to metabolize aromatic hydrocarbons to afford synthetically useful chiral synthons of type **2** (Scheme 1-1).<sup>29</sup> With the disclosure of racemic pinitol synthesis by Ley in 1987,<sup>30</sup> the practical utility of TDO in synthesis



Scheme 1-1. Aromatic degradation pathway of *Pseudomonas putida* F39D

was realized. Since then, metabolites obtain through biooxidation of aromatic substrates with *P. putida* F39D have been used in various enantioselective syntheses, including polyphenylene,<sup>31,32</sup> prostaglandin E<sub>2a</sub>,<sup>33-35</sup> and indinavir<sup>36,37</sup> that are of industrial and medical significant.

The historical chapter of this dissertation gives some important developments in the realm of biotransformation. The use of molecular oxygen as oxidizing agent by monooxygenases and dioxygenases-carrying microorganisms for the conversion of alkanes, alkenes, and aromatic to more polar identities is also discussed. A section of this chapter is designated for a brief review of TDO properties, including the enzyme's substrate specificity, regioselectivity, and stereoselectivity. Selected syntheses of highly oxygenated natural products and targets of industrial significant are highlighted to

illustrate the versatility as well as the practicality of TDO as a biocatalyst for synthesis. The syntheses of a few substituted catechols by conventional chemical methods as well as by enzymatic means are included. The last part of the historical chapter is saved for reviewing a class of natural products, combretastatins, including their discovery, biological activities, and syntheses because combretastatins A-1 and B-1 are among the synthetic targets of this work.

The results and discussion chapter of this document is devoted to explore the synthetic utility of toluene dioxygenase (TDO) and the second enzyme, toluene *cis*-dihydrodiol dehydrogenase (TDDH), of *Pseudomonas putida* F39D through gaining better understanding of the enzymes' properties and functions. The kinetic resolution capacity of TDO in resolving aromatic substrates with proximal and distal stereogenic centers and the possibility of the enzyme going through a radical intermediate during the catabolism of aromatic substrates are among the main topics of this chapter. The utility of TDDH to generate substituted catechols also will be discussed as an environmental benign method to generate synthons for approaches to combretastatins A-1 and B-1. The synthesis of polyhydroxylated chiral polymer is the last part of this chapter.

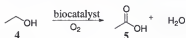
## CHAPTER 2 HISTORICAL

### **Biotransformations**

Throughout the history of mankind, microorganisms have been of tremendous social and economic importance. Without us even being aware of their existence, microorganisms were used in food and beverage production very early in history. Sumerians and Babylonians practiced beer brewing before 6000 B.C. Egyptians, Hebrews, Greeks, and later Gauls and Iberians, began using yeast to produce fermented food products such as bread, wine, and beer as early as 4000 B.C. It was only in the 19th century that scientific progress revealed the secrets behind yeast fermentation. The pioneering work of French chemist, Louis Pasteur, in the mid-1800s, led to a scientific understanding of yeast fermentation that eventually became industrially important in producing chemical commodities such as alcohols and organic material by fermentation. Lactic acid was probably the first optically active compound to be produced industrially by fermentation, in 1880.<sup>38</sup>

In the course of time, it was discovered that microorganisms could modify certain compounds by simple, chemically well-defined reactions catalyzed by enzymes.<sup>39</sup> Today, these processes are termed "biotransformations." The essential difference between fermentation and biotransformation is that there are several catalytic steps between substrate and product in fermentation; while there are only one or two steps in biotransformation. Another distinction is that the chemical structures of the substrate and the product are similar in biotransformation, but not necessarily in fermentation.

The story of microbial biotransformations is closely connected with vinegar



Scheme 2-1. Vinegar production

production, dating back to 2000 years B.C. Vinegar production, perhaps the oldest example of microbial oxidation, shows important developments in the field of biotransformation by living cells (Scheme 2-1). However, much of the scientific evidence for this important microbial transformation did not emerge until the mid-1800s.

The ability of a microbe to carry out kinetic resolution of racemic compounds was first recognized by Pasteur in 1858 when ( $\pm$ )-tartaric acid was resolved with *Penicillium*

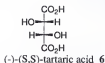


Figure 2-1. Pasteur's product of the first resolution reaction.

*glaucum*.<sup>40</sup> He showed that the organism resolved racemic tartaric acid by consuming only the (+)-tartaric acid, while leaving the (-)-enantiomer 6 untouched (Figure 2-1).<sup>41</sup> From his work in this area, Pasteur became interested in fermentation and the concept of spontaneous generation, a widely held belief that living organisms can somehow arise spontaneously from nonliving matter.

Over the next few years, Pasteur became deeply involved with the French wine industry investigating the "l'amer" condition, which turned wine sour, and destroyed large quantities of the best Burgundy every year. At the time, fermentation was thought to be a straightforward chemical breakdown of sugar into alcohol, carbon dioxide and water (as a result of inherent instability of sugar). Scientists had found yeast cells in fermenting vats of wines and recognized them as being living organisms, but believed

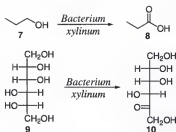
they were a product of fermentation. During his investigation of the "l'amer" condition for the French wine industry, Pasteur examined specimens of wines under a microscope and saw plump yeast cells in good wine and rod-like shapes in wine with too much acid. Analysis of the wine showed that organic, or biological compounds were formed during the fermentation, products that sugar alone could not produce. From his previous research, Pasteur knew these compounds had to be formed by living cells. Pasteur realized that the fermentation process was conducted by yeast and that other microorganisms were responsible for turning the fermentation sour.

Pasteur eventually identified and isolated specific microorganisms responsible for normal and abnormal fermentations of wine, beer and vinegar, and gave birth to the science of bacteriology. He also showed that by heating each of the above liquids, the microorganisms were killed and the wine was protected from further contamination. With the identification of undesirable bacterial contamination of wines, volatile acidity was eliminated and better quality wines were more routinely produced.

Pasteur's pioneer work in fermentation in 1862 conclusively disproved the theory of spontaneous generation and led to the germ theory of infection. His work on wine, vinegar, and beer resulted in the development of the pasteurization process.

In 1886, Brown confirmed Pasteur's findings and named *Bacterium xylinum* as the agent responsible vinegar production.<sup>42</sup> He also found that this organism could oxidize propanol **7** to propionic acid **8** and mannitol **9** to fructose<sup>43</sup> **10** (Scheme 2-2).

In 1897, Eduard Buchner,<sup>44</sup> a German chemist who also had been interested in the problems of alcoholic fermentation since the 1880s, argued that enzymes were the

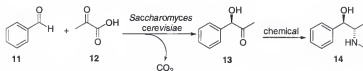


Scheme 2-2. Reaction catalyzed by *Bacterium xylinum*

causative agents responsible for conversion of alcohol to acid and not whole yeast cells as was originally thought. This view was not in line with the conventional belief at that time, namely, that without life there could be no fermentation. Despite of this, Buchner and his brother Hans Buchner in 1897 succeeded in preparing a cell-free liquid extract that was capable of converting sugar into ethanol and carbon dioxide, just as though whole yeast cells had been present. He named the causative agent as zymase.<sup>45</sup> Buchner succeeded in the proving this concept where many others, including Liebig and Pasteur, had failed. He did this by grinding yeast cells with sand and then filtering the crude preparation for enzyme isolation. Eventually, it became clear that the conversion of sugar into alcohol by means of yeast juice entails a series of stepwise reactions, and that zymase really is a mixture of several enzymes. Buchner's discovery of zymase then led to establishment of the enzyme theory of fermentation. This gave birth to the science of biochemistry, in which where much effort over the years has been invested in gaining a thorough understanding of biological systems.

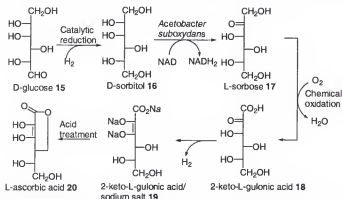
As biochemical knowledge of microorganisms and their environmental function become more evident, microorganisms are being used more frequently for the production of fine chemicals. Neuberg and Hirsch<sup>46,47</sup> in 1921 showed that acyloin condensation<sup>20-</sup>

<sup>22,48</sup> of benzaldehyde **11** and 2-oxo-propionic acid **12** could be achieved to furnish levorotatory 1-hydroxy-1-phenylpropan-2-one **13**, when the substrates are added to a carbohydrate solution undergoing active yeast fermentation. This process was later modified to use acetaldehyde directly instead of 2-oxo-propionic acid to afford a much better yield of  $\alpha$ -hydroxy ketone **13** which is used industrially in the production of L-ephedrine **14**<sup>49,50</sup> (Scheme 2-3).



Scheme 2-3. *Saccharomyces cerevisiae* catalyzes acyloin condensation as the key step in the production of L-Ephedrine

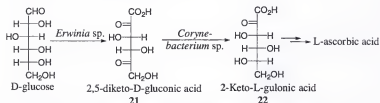
The ability of microorganisms to carry out biotransformations such as acyloin condensation mentioned above, was soon recognized as essential in the manufacture of



Scheme 2-4. Microbial dehydrogenation of D-sorbitol to L-sorbose as the key step in the Reichstein-Grüssner synthesis of L-ascorbic acid

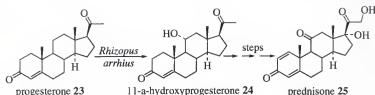
many biologically active compounds. The ability of *Acetobacter suboxydans*<sup>51</sup> to oxidize D-sorbitol **16** to L-sorbose **17** was discovered in 1928 and used as the key step in the Reichstein-Grussner synthesis of L-ascorbic acid **20** in 1934<sup>52</sup> (Scheme 2-4). The importance of L-ascorbic acid in the production of vitamins and its use as an antioxidant in the manufacture of food accounts for its worldwide production of about 40,000 tons each year.

Today, in addition to the Reichstein-Grussner synthesis (Scheme 2-4), the commercial production of L-ascorbic acid is carried out using *Erwinia* sp. and *Coryne bacterium* sp. in two fermentation steps to provide approximately 1 kg of L-ascorbic acid for every 2 kg of glucose used (Scheme 2-5).<sup>53</sup>

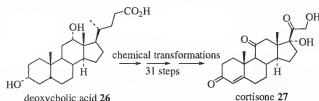


Scheme 2-5. Two-stage fermentation for the production of 2-keto-L-gulonic acid from D-glucose

Another industrially important microbial transformation was disclosed in 1952<sup>54,55</sup> for the conversion of that progesterone **23** to 11- $\alpha$ -hydroxyprogesterone **24** by the molds *Rhizopus arrhizus*.<sup>56</sup> The significance of this microbial transformation was soon realized when the hydroxylated metabolite **24** was used in the synthesis of therapeutic steroids and their derivatives.<sup>57</sup> The microbial hydroxylation step simplified and improved the efficiency of many approaches to synthesis of corticosteroid hormones and their derivatives. An example of such an application is illustrated in the production of prednisone **25**, from 11-hydroxyprogesterone **24** (Scheme 2-6). Although the

Scheme 2-6. Microbial 11- $\alpha$ -hydroxylation of progesterone

chemical syntheses of similar steroids were available from deoxycholic acid 26, they were deemed lengthy, complicated and uneconomical, as in the case of cortisone 27 synthesis, which involves 31 steps (Scheme 2-7).<sup>58</sup> The microbial 11- $\alpha$ -hydroxylation



Scheme 2-7. Chemical synthesis of cortisone

of progesterone quickly made the commercialization of steroid derivatives such as cortisone a reality by reducing the price of this commodity from \$200 to \$6 per gram.

Table 2-1. Examples of commercial steroid processes

| Substrate                   | Product                                         | Microorganism                                             | Manufacturer                          |
|-----------------------------|-------------------------------------------------|-----------------------------------------------------------|---------------------------------------|
| Progesterone                | 11 $\alpha$ -Hydroxy-progesterone               | <i>Rhizopus nigricans</i>                                 | Upjohn company                        |
| Component S                 | Cortisol                                        | <i>Curvularia lunata</i>                                  | Pfizer, Inc.,<br>Gist-Brocades        |
| 9 $\alpha$ -Fluoro-cortisol | 9 $\alpha$ -Fluoro-16 $\alpha$ -hydroxycortisol | <i>Streptomyces roseochromogens</i>                       | E. R. Squibb & Sons                   |
| Cortisol                    | Prednisolone                                    | <i>Arthrobacter simplex</i>                               | Lederle Laboratories                  |
| Progesterone                | 1-Dehydro-testololactone                        | <i>Cylindrocarpus radiculicola</i>                        | Schering Corp.<br>E. R. Squibb & Sons |
| $\beta$ -Sitosterol         | Androstadiendione                               | <i>Mycobacterium sp.</i> ,<br><i>M. fortuitum mutants</i> | G. D. Searle & Co.<br>Upjohn company  |

Further improvements of this biotransformation process have led to a current price of less than \$1 per gram of cortisone.<sup>39</sup>

The microbial reaction steps listed in Table 2-1 are of great economic significance for the commercial production of steroids. For example, the transformation of progesterone to a C19-steroid is used industrially in testosterone and estrogen production and the microbial dehydration of ring A is also used in estrogen production.<sup>57</sup>

In the 1950s, the DNA double helix structure – the genetic code of heredity – and the chemical nature of RNA were discovered. This discovery can be regarded as a major milestone among this century's main scientific achievements. It led to the synthesis of recombinant DNA and gave impetus to genetic engineering in the sixties and seventies. This development quickly made recombinant DNA technology an integral part of

Table 2-2. Some representative industrial whole-cells biotransformations.

| Products                            | Biocatalyst                        | Since | Company                          |
|-------------------------------------|------------------------------------|-------|----------------------------------|
| Vinegar                             | bacteria                           | 1823  | Various                          |
| L-2-Methylamino-1-phenylpropan-1-ol | yeast                              | 1930  | Knoll AG, Germany                |
| L-Sorbose                           | <i>Acetobacter suboxydans</i>      | 1934  | Various                          |
| Prednisolone                        | <i>Arthrobacter simplex</i>        | 1955  | Schering AG, Germany             |
| L-Aspartic acid                     | <i>Escherichia coli</i>            | 1958  | Tanabe Seiyaku Co., Japan        |
| 7-ADCA                              | <i>Bacillus megaterium</i>         | 1970  | Asahi Chemical Industry, Japan   |
| L-Malic acid                        | <i>Brevibacterium ammoniagenes</i> | 1974  | Tanabe Seiyaku Co., Japan        |
| D-p-Hydroxy-phenylglycine           | <i>Pseudomonas striata</i>         | 1983  | Kanegafuchi, Chemical Co., Japan |
| Acrylamide                          | <i>Rhodococcus sp.</i>             | 1985  | Nitto Chemical Ltd, Japan        |
| D-Aspartic acid and L-Alanine       | <i>Pseudomonas dacunhae</i>        | 1988  | Tanabe Seiyaku Co., Japan        |
| L-Carnitine                         | <i>Agrobacterium sp.</i>           | 1993  | Lonza, Czech. Rep.               |
| 2-Keto-L-gluconic acid              | <i>Acetobacter sp.</i>             | 1999  | BASF, Merck, Cerestar, Germany   |

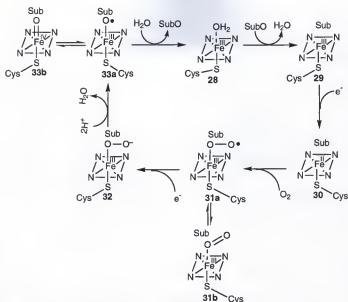
industrial microbial transformation. Recombinant technology which is widely used today, allows over-expression of desired proteins such as biocatalysts, thereby resulting in higher yields and diminished biological waste. Almost all chemical and pharmaceutical companies rely on one or more forms of this technology to meet the challenges of their trade. Table 2-2 lists some representative industrial whole-cells biotransformations for the production of specialty chemicals.<sup>60</sup>

### **Oxygenation Reactions**

Molecular oxygen is a powerful oxidizing agent essential for life's functions in aerobic organisms. Oxygen interacts with a variety of biological systems including oxidases, peroxidases and oxygenases that are important in cellular energy metabolism and respiration.<sup>61</sup> These enzymes utilize transition metals, most commonly Fe and Cu, or heteroaromatic systems such as pteridium<sup>62</sup> or flavin to activate dioxygen into highly reactive singlet and hydroxyl radical species for electron transfer and oxidation of organic molecules.

One of the most widely distributed enzyme systems among living organisms that utilize oxygen is the iron-dependent cytochrome P-450 monooxygenases.<sup>63-66</sup> These enzymes are particularly abundant in detoxification organs such as the liver, but they are also found in microorganisms. This class of enzymes was discovered in 1947<sup>67,68</sup> and has been found to be involved in a variety of biochemical pathways including steroid metabolism,<sup>69-71</sup> assimilation of non-carbohydrate carbon sources<sup>72,73</sup> and detoxification of xenobiotics. Numerous studies have been devoted to this enzyme system and the cyclic mechanism for P-450 catalyzed oxygenation reactions was first postulated in 1971,<sup>74</sup> and the crystalline structure of the enzyme has been elucidated in the presence<sup>75</sup>

and absence of substrate.<sup>76</sup> A summary of the catalytic cycle catalyzed by cytochrome P-450 is depicted in Scheme 2-8.



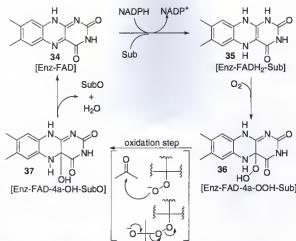
Scheme 2-8. Monooxygenase cytochrome P450s catalytic cycle

In the resting stage, the iron species is coordinated equatorially by a heme moiety with water or hydroxide as an exchangeable axial ligand trans to the thiolate of a cysteine residue, **28**. The binding of substrate is accompanied by the desolvation of the binding pocket and expulsion of the quao axial ligand<sup>77</sup> to give rise to species **29**. This process alters the enzyme's configuration and increases its redox potential by 130 mV.<sup>78</sup> These changes allow the enzyme to accept an electron from NAD(P)H to give species **30** onto which a molecular oxygen is bound to give a Cytochrome P-450 dioxygen complex that can be represented by two resonances forms, **31a** and **31b**. The addition of a second

electron to **31** yields a ferric peroxide adduct **32**, which can be protonated to give a hydroperoxide complex. A second protonation of the same oxygen then leads to heterolytic O-O bond cleavage, releasing water and generating the proposed oxo-ferryl ( $\text{O}=\text{Fe}^{\text{IV}}$ ) porphyring radical **33a** intermediate that is sometimes represented as  $(\text{FeO})^{3+}$  **33b**. One atom of oxygen is expelled as water while the other forms the reactive intermediate  $(\text{FeO})^{3+}$  which oxidizes the substrate to form the product. The expulsion of the product, SubO, regenerates the initial ferric iron porphyrin species<sup>79</sup> and closes the catalytic cycle. The mechanistic representation of Cytochrome P-450 above is illustrative of how an enzyme system can utilize oxygen in the catabolism of organic matter.

In contrast, flavin-dependent mono-oxygenases use a different mechanism, which involves a flavin cofactor, Scheme 2-9.<sup>80-82</sup> First, NADPH reduces the Enz-FAD complex thus destroying aromaticity. The  $\text{FADH}_2$  so formed is oxidized via Michael-type addition of molecular oxygen yielding a hydroperoxide species ( $\text{FAD-4a-OOH}$ ). Deprotonation of the latter affords a peroxide anion, which undergoes a nucleophilic attack on the carbonyl group of the substrate, usually an aldehyde or a ketone. The tetrahedral intermediate thus formed, collapses via rearrangement of the carbon-framework forming the product ester or lactone. Finally, water is eliminated from the  $\text{FAD-4a-OH}$  species to reform FAD.

The cytochrome P450 mechanism resembles the chemical oxidation by hypervalent transition metal oxidants through an electrophilic attack, while the FAD-dependent mechanism parallels the oxidation of organic compound by peroxides or peracids, which act as nucleophiles.<sup>83</sup>



Scheme 2-9. Catalytic cycle of flavin-dependent mono-oxygenases

Enzymatic oxygenation reactions are particularly intriguing since direct oxyfunctionalization of unactivated organic compounds are difficult to attain through conventional chemical methods. Thus transformations of cheap inert raw materials, such as alkanes, alkenes and aromatics into oxidized products of greater value such as aldehydes, ketones, alcohols, carboxylic acids, by means of biocatalysts are appealing alternatives to industry. The highly regio- and enantiospecific properties of many biocatalyst systems made them ideal for the precise manufacturing of specialty chemicals. The enzymatic oxygenation of organic molecule may go through one of the following processes depending upon the enzyme system involved, Scheme 2-10.

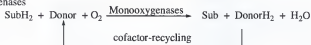
In reactions catalyzed by monooxygenases, one oxygen atom from molecular oxygen is incorporated into the substrate and the other one is reduced to form water by means of a cofactor usually NADH or NADPH. While in reactions catalyzed by dioxygenases, the enzymes simultaneously incorporate both oxygen atoms into the

substrate. These two processes are among the most common and important in aromatic hydrocarbon oxidation.

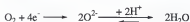
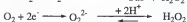
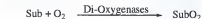
#### Dehydrogenases



#### Oxygenases



#### Oxidases



#### Peroxidases

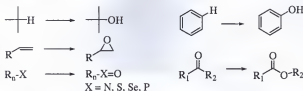
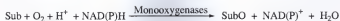


Scheme 2-10. Enzymatic oxidation reactions

### Monooxygenases Catalyze Biotransformation

Monooxygenases are ubiquitous in nature and serve many important biological functions including detoxification of xenobiotics. They have a broad range of substrates and can tolerate a very wide range of functional groups (Scheme 2-11).

Although the reaction mechanisms of various monooxygenases differ greatly depending upon the sub-type of enzyme, their mode of oxygen-activation is the same in term of their requirement of NAD(P)H and oxygen for function. In the following sections, a series of monooxygenases that catalyze the mono-hydroxylation of alkanes, arenes, and alkenes as well as enzymes that performed Baeyer-Villiger reactions are described.



| Substrate               | Product          | Type of reaction     | Type of cofactor |
|-------------------------|------------------|----------------------|------------------|
| alkane                  | alcohol          | hydroxylation        | metal-dependent  |
| aromatic                | phenol           | hydroxylation        | metal-dependent  |
| alkene                  | epoxide          | epoxidation          | metal-dependent  |
| ketone                  | ester/lactone    | Baeyer-Villiger      | flavin-dependent |
| heteroatom <sup>a</sup> | heteroatom-oxide | heteroatom oxidation | flavin-dependent |

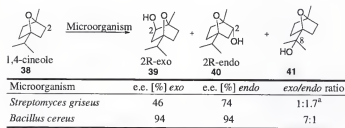
Scheme 2-11. Monooxygenase catalyzed reactions

### Hydroxylation of Alkanes

Hydroxylation of unactivated hydrocarbon is a subject of great interest within the synthetic community.<sup>84-86</sup> However, despite the invested effort, functionalization of unactivated hydrocarbon remains an unresolved challenge. Available approaches to the problem are limited<sup>87-89</sup> and problematic with little regio- and stereoselectivity control.

By contrast, biochemical hydroxylation of unactivated hydrocarbon is common and is among the most useful processes<sup>90-94</sup> with great synthetic utility. Biooxidation of the hydrocarbon of steroids and terpenoids, for example, are industrially important processes. In this aspect, methane oxygenases play many important roles. This class of enzyme specializes in the conversion of alkane to alcohol in the presence of NADH.<sup>95</sup> The enzymes from two obligate methylotrophs, *Methylococcus capsulatus* (Bath)<sup>96,97</sup> and *Methylosinus tricosporium* OB3b<sup>98</sup> have been isolated, purified, and studied. They have been shown to have a very broad substrate range and are capable of oxidizing a variety of

alkanes, alkenes, aromatic, and heterocyclic compounds,<sup>99</sup> sometimes with little regioselectivity control. Complex substrates have been converted into more than one oxidation product as in the case of hydroxylation of 1,4-cineole **38** by *Streptomyces griseus*<sup>100</sup> and *Bacillus cereus*<sup>101</sup> (Scheme 2-12). When *Streptomyces griseus* was used, 8-hydroxycineole **41** was observed to be the major product along with trace amounts of *exo*- and *endo*-2-hydroxy derivatives with low optical purity. On the other hand, when *Bacillus cereus* was used, (2*R*)-*exo*- and (2*R*)-*endo*-hydroxycineoles were obtained, exclusively in a ratio of 7:1, both in excellent enantiomeric excess.



<sup>a</sup> 8-hydroxycineole was the major product

Scheme 2-12. Microbial hydroxylation of 1,4-cineole

However there are monooxygenase systems that are extremely precise in regioselectivity and give rise to only one oxidation product. The importance of such biocatalyst systems have been realized and used industrially for hydroxylating of the 9 $\alpha$ - and 16 $\alpha$ -carbons the steroid framework in the production of therapeutic agents.<sup>102,103</sup> Today, almost all carbon centers of the steroid skeleton can be selectively hydroxylated by using an appropriate microorganism.<sup>104</sup> For example, the 11 $\alpha$ -hydroxylation of progesterone **42** can be achieved with *Rhizopus arrhizus*<sup>55</sup> or *Aspergillus niger*<sup>105</sup> and is used industrially in manufacturing of 11 $\alpha$ -hydroxyprogesterone (Figure 2-2). The 7 $\beta$ -hydroxylation of lithocholic acid **43** via *Fusarium equiseti*<sup>106</sup> is another good example of

methane oxygenase transformation to generate drugs capable of dissolving cholesterol and which also can be used for therapeutic treatment of gallstones (Figure 2-2).

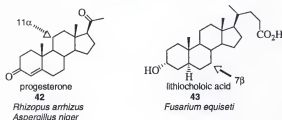


Figure 2- 2. Microbial hydroxylation of steroids

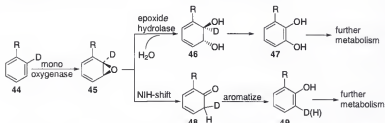
### Hydroxylation of Arenes

Aromatic hydrocarbons are stable identities and are notoriously inert to most chemical manipulations. Harsh conditions, which are often associated with low selectivity and uncontrollable formation of byproducts, are often required for functionalization of the aromatic moiety. The difficulties associated with such methods make them unappealing and impractical for synthetic applications.<sup>107-109</sup>

By contrast, selective hydroxylation of aromatic compounds can be achieved through enzymatic means. Monohydroxylation of aromatic substrates by monooxygenases has been the subject of many studies. Most monooxygenases require NAD(P)H to reduce the flavin to a 4 $\alpha$ -hydroxyperoxyflavin required for hydroxylation of the aromatic ring. The transformation is generally very regioselective and the hydroxylating sites tend to be either *ortho*-<sup>110,111</sup> or *para*-<sup>112,113</sup> to the existing substituent of the aromatic ring. *Meta*-hydroxylation on the other hand is rarely observed.<sup>114</sup>

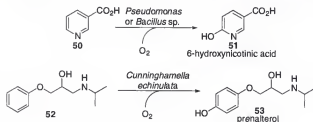
Mechanistic studies of monooxygenases in eukaryotic cells such as fungi, yeasts and higher organisms suggested that the process proceeds predominantly via epoxidation of the aromatic species to generate unstable arene-oxides 45 as the first step in the

biotransformation process. The instability of arene-oxides has been shown to be responsible for toxic and mutagenic effect by reacting with DNA, RNA and proteins.<sup>115</sup> Arene-oxide can rearrange easily via hydride anion (NIH-shift)<sup>116</sup> migration to form the phenolic product<sup>117</sup> **49** or hydrolyzed to a *trans*-diol **46** by epoxide hydrolase (Scheme 2-13). Phenolic components are often further oxidized by polyphenol oxidases to corresponding catechols<sup>118</sup> which can undergo further metabolism.



Scheme 2-13. Possible mechanism in biodegradation of aromatics by eukaryotic cells

Enzymatic hydroxylation of aromatic substrates is a well-established industrial process for the production of therapeutic agents. The production of L-DOPA (3,4-dihydroxy-phenyl alanine)<sup>119</sup> for treatment of Parkinson's Disease<sup>120</sup> and 6-hydroxynicotinic acid **51** from nicotinic acid by either *Pseudomonas* or *Bacillus* sp. are just two examples of monooxygenase-catalyzed aromatic hydroxylation reactions used



Scheme 2-14. Microbial hydroxylation of aromatics

industrially (Scheme 2-14). The production of the pharmacologically important  $\beta$ -blocker, racemic prenalterol **53**, via selective *p*-hydroxylation of a simple aromatic precursor, using *Cunninghamella echinulata*,<sup>121</sup> is another good example of whole-cell biotransformation.<sup>122-126</sup>

### Epoxidation of Alkenes

Chiral epoxides are extensively employed as high-value intermediates in the synthesis of chiral compounds. In recent years much research has been devoted to the development of catalytic methods for their production.<sup>127</sup> The Katsuki-Sharpless methods for the asymmetric epoxidation of allylic alcohols<sup>128</sup> and the asymmetric dihydroxylation of alkenes are now widely applied as reliable procedures. Catalysts for the epoxidation of non-functionalized olefins have been developed more recently.<sup>129,130</sup> Although high selectivity has been achieved for the epoxidation of *cis*-alkenes, the selectivity achieved with *trans*- and terminal olefins were less selective and continue to be a challenge within the synthetic community.

By contrast, monooxygenases-catalyzed epoxidation can be achieved with excellent stereoselectivity from a wide range of alkenes that are not accomplishable via traditional methods.<sup>131,132</sup> Despite the wide distribution of mono-oxygenases within all types of organisms, only alkane and alkene utilizing bacteria, and to a lesser extent fungi, have been shown to be capable of epoxidizing alkenes.<sup>4,91,133</sup>

The most intensively studied microbial epoxidizing agent is the  $\omega$ -hydroxylase system of *Pseudomonas oleovorans*.<sup>134</sup> This consists of three protein components: (i) rubredoxin, (ii) NADH-dependent rubredoxin reductase and (iii) an  $\omega$ -hydroxylase (a sensitive non-heme iron protein), and catalyzes not only the hydroxylation of aliphatic C-H bonds, but also the epoxidation of alkenes.<sup>135</sup>

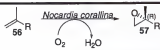
Besides *Pseudomonas oleovorans*, numerous bacteria have been shown to epoxidize alkenes.<sup>136</sup> As shown in Table 2-3, the optical purity of epoxides depends upon the strain used, although the absolute configuration is usually R.<sup>137</sup> This concept has been recently applied to the synthesis of chiral alkyl and aryl glycidyl ethers.<sup>138,139</sup> The latter are of interest for the preparation of 3-substituted 1-alkylamino-2-propanols, which are widely used as adrenergic receptor blocking agents.<sup>140</sup>

Table 2-3. Microbial epoxidation of alkenes

| Microorganism                 | R <sub>1</sub>                                                                     | R <sub>2</sub>  | Config. | e.e. <sup>(89/9)</sup> |
|-------------------------------|------------------------------------------------------------------------------------|-----------------|---------|------------------------|
| <i>Pseudomonas oleovorans</i> | n-C <sub>5</sub> H <sub>11</sub>                                                   | H               | R       | 70-80                  |
|                               | n-C <sub>7</sub> H <sub>15</sub>                                                   | H               | R       | 60                     |
|                               | H                                                                                  | H               | R       | 86                     |
|                               | NH <sub>2</sub> CO-CH <sub>2</sub> -C <sub>6</sub> H <sub>4</sub> -O               | H               | S       | 97                     |
|                               | CH <sub>3</sub> O(CH <sub>2</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>4</sub> -O | H               | S       | 98                     |
| <i>Corynebacterium equi</i>   | CH <sub>3</sub>                                                                    | H               | R       | 70                     |
|                               | n-C <sub>13</sub> H <sub>27</sub>                                                  | H               | R       | ~100                   |
| <i>Mycobacterium</i> sp.      | H                                                                                  | H               | R       | 98                     |
|                               | CH <sub>3</sub>                                                                    | CH <sub>3</sub> | R,R     | 74                     |
|                               | Ph-O                                                                               | H               | S       | 80                     |
| <i>Xanthobacter</i> Py2       | Cl                                                                                 | H               | S       | 98                     |
|                               | CH <sub>3</sub>                                                                    | CH <sub>3</sub> | R,R     | 78                     |
| <i>Nocardia</i> sp. IP1       | Cl                                                                                 | H               | S       | 98                     |
|                               | CH <sub>3</sub>                                                                    | H               | R       | 98                     |

More recently, other microorganisms also have been found to perform epoxidation of alkenes with excellent yield and optical purity. As can be seen from Table 2-4, non-terminal alkenes can be epoxidized by a *Mycobacterium* or *Xanthobacter* sp.,<sup>141</sup> while *Nocardia corallina* has been reported to convert branched alkenes into the corresponding (*R*)-epoxides with good optical purities.

Table 2-4. Epoxidation of branched alkenes by *Nocardia corallina*

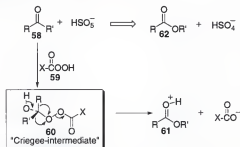
|  |          |
|-----------------------------------------------------------------------------------|----------|
| R                                                                                 | e.e. [%] |
| n-C <sub>3</sub> H <sub>7</sub> -                                                 | 76       |
| n-C <sub>4</sub> H <sub>9</sub> -                                                 | 90       |
| n-C <sub>5</sub> H <sub>11</sub> -                                                | 88       |

However, the disadvantages associated with mono-oxygenase catalyzed epoxidation reactions include the enzyme's instability, its dependency upon cofactors and the precise coordination requirement of its subunits for proper function. These factors discourage the exploitation of isolated enzymes as biocatalysts for epoxidation of alkenes. Instead, whole-cell fermentations are generally employed. The drawback of this approach is often linked to the epoxides tendency to react with cellular proteins and enzymes leading to cell death. If the problem of product toxicity is surmounted, the microbial epoxidation of alkenes also will be feasible on an industrial scale.<sup>142,143</sup> The toxic effects of the epoxide formed and its further (undesired) metabolism by epoxide hydrolases can be reduced by employing biphasic media<sup>144</sup> for the production of chiral epoxides. Despite such difficulties, enzymatic epoxidation of alkenes continues to be a reliable alternative for numerous synthetic applications, an example of which is the generation of the epoxy-phosphonic acid derivative, 'fosfomycin',<sup>145</sup> whose enantiospecific synthesis by classical methods would have been extremely difficult. This was accomplished by a microbial epoxidation reaction using *Penicillium spinulosum*.

### Baeyer-Villiger Reactions

The Baeyer-Villiger oxidation of ketone with a peroxy acid to produce corresponding lactone/ester was first reported by Baeyer and Villiger over a century

ago.<sup>146</sup> Fifty years later, the mechanism of this reaction was deciphered by Criegee<sup>147</sup> and is still accepted in its original formulation (Scheme 2-15). The reaction proceeds via



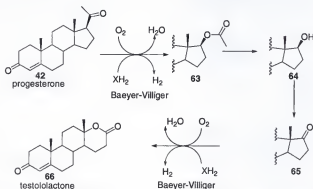
Scheme 2-15. Mechanism of the Baeyer-Villiger reaction

the formation of an intermediate resulting from addition of the peracid to the carbonyl group to form the tetrahedral "Criegee-intermediate" **60** followed by migration of one of the substituents on the peroxy oxygen and formation of the product. The migration step is concerted, rate determining and product determining. The regiochemistry of oxygen-insertion of the chemical and the enzymatic Baeyer-Villiger reaction can usually be predicted by assuming that the carbon atom best able to support a positive charge will migrate preferentially.<sup>148</sup> The applications of the Baeyer-Villiger reaction in organic synthesis are widespread and have been reviewed.<sup>91,133,148-152</sup>

In biological systems, the Baeyer-Villiger reaction is carried out by monooxygenases. Enzymatic Baeyer-Villiger oxidation is a flavin-dependent process that catalyzes the breakdown of carbon structures containing a ketone moiety. Mechanistically, the process is closely related to the purely chemical process<sup>80,153</sup> proposed by Griegee. Of the enzymes studied to date, almost without exception all have been found in bacteria and fungi.<sup>154</sup>

A number of bacterial enzymes have been purified and characterized,<sup>155</sup> however, the best characterized Baeyer-Villiger Monooxygenase (BVMO) to date is cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus*. The complete sequence of the enzyme is available,<sup>156</sup> and the enzyme is believed to be monomeric with only one active site.<sup>157</sup>

The importance of BVMOs was first recognized over 40 years ago during the initial era of steroid biotransformation when the biotransformation of progesterone **42** by

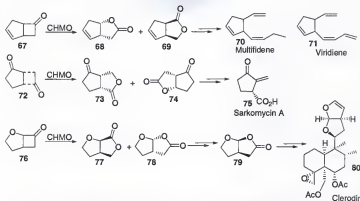


Scheme 2-16. Progesterone metabolism by BVMOs in *Cylindrocarpon radicicola*

*Cylindrocarpon radicicola*<sup>158</sup> was used in the synthesis of testolactone **66** (Scheme 2-16). Over the intervening years, the scope of the reaction was vigorously studied. The enzyme is found to be capable of oxidizing a variety of substrates with excellent regio- and stereoselectivity,<sup>159-161</sup> making it an ideal synthetic tool for chemoenzymatic approaches to synthesis of a wide range of useful compounds.<sup>162</sup>

Biotransformations of various unsubstituted bicyclic ketones containing a furan or pyran ring have been used to yield chiral synthons for chemoenzymatic syntheses of

useful compounds, such as multifidene **70** and viridiene **71**,<sup>163</sup> the antibiotic sarkomycin A **75**,<sup>164</sup> and the insect antifeedant clerodin **80** (Scheme 2-17).<sup>165</sup>



Scheme 2-17. Chemoenzymatic syntheses of various useful target molecules involving the biotransformation by CHMO of bicyclo[3.2.0]-type ketones

BVMOs are also an excellent source of catalyst for desymmetrization of prochiral ketones to yield the corresponding lactones.<sup>17,166</sup> As depicted in Table 2-5, CHMO catalyzed oxygen insertion occurred on both sides of the ketone, depending upon the

Table 2-5. Enzymatic Baeyer-Villiger oxidation of prochiral ketones

|                     |               |               |
|---------------------|---------------|---------------|
| <br><b>81</b>       | <br><b>82</b> | <br><b>83</b> |
| R                   | Configuration | e.e. [%]      |
| CH <sub>3</sub> -O- | S             | 75            |
| Et-                 | S             | >98           |
| n-Pr-               | S             | >98           |
| t-Bu-               | S             | >98           |
| n-Bu-               | R             | 52            |

substituent in the 4-position, to give predominantly the (S)-configuration products. A switch to the R-lactone was observed with 4-*n*-butylcyclohexanone. Simple models were recently developed which allow the prediction of the stereochemical outcome of Baeyer-Villiger oxidation catalyzed by cyclohexanone mono-oxygenase of *Acinetobacter* and *Pseudomonas* sp by determining which group within the Criegee-intermediate is prone to migration.<sup>167,168</sup>

Table 2-6. Microbial Baeyer-Villiger oxidation of monocyclic ketones

|                                           |                     |  |
|-------------------------------------------|---------------------|--|
|                                           |                     |  |
| R                                         | e.e. of lactone [%] |  |
| <i>n</i> -C <sub>5</sub> H <sub>11</sub>  | 97                  |  |
| <i>n</i> -C <sub>7</sub> H <sub>15</sub>  | 95                  |  |
| <i>n</i> -C <sub>9</sub> H <sub>19</sub>  | 85                  |  |
| <i>n</i> -C <sub>11</sub> H <sub>23</sub> | 73                  |  |

Racemic ketones can be resolved by BVMOs, in which only one enantiomer gets oxidized and its counterpart remains unchanged,<sup>169</sup> Table 2-6. For example,  $\alpha$ -substituted cyclopentanones were stereospecifically oxidized by an *Acinetobacter* sp. to form the corresponding (S)-configured  $\delta$ -lactones,<sup>170</sup> substances which constitute valuable components of various fruit flavors.

Bicyclic halo-ketones, which are used for the synthesis of anti-viral 6'-fluoro-carbocyclic nucleoside analogues, were resolved by using the same technique.<sup>171</sup> Both enantiomers were obtained with >95% optical purity. The exquisite enantioselectivity of the microbial oxidation is due to the presence of the halogen atoms, since the de-halogenated bicycle[2.2.1]hepan-2-one was transformed with low selectivity.

Above-mentioned Baeyer-Villiger oxidations are not trivial to perform on a preparative scale. Whole-cell transformations are difficult to perform, as some strains such as *Acinetobacter calcoaceticus* are pathogenic and suffer from low yields due to over-metabolism and/or side reactions catalyzed by other active enzymes in the microorganisms. On the other hand, the use of isolated mono-oxygenases for Baeyer-Villiger oxidations is not trivial, as these enzymes are linked to NADPH-recycling, which is a notoriously difficult process.

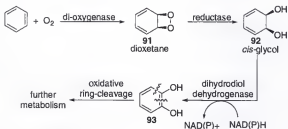
The difficulties posed by BVMOs have led investigators to seek other looked elsewhere for alternatives. In this context, using yeast genetically engineered to contain the cyclohexanone monooxygenase of *Acinetobacter calcoaceticus*, has been an excellent and safe substitute for preparative applications.<sup>172-175</sup>

### Dioxygenases Catalyze Biotransformation

The benzene nucleus originating from both natural and anthropogenic sources is one of the most abundant chemical structures in the biosphere. This class of compounds has attracted a lot of attention from the scientific communities due to its adverse health affects. The relationship between cancer and exposure to this class of chemical was first suggested in 1761<sup>176</sup> and further supported by evidence presented in 1775,<sup>177</sup> showing a high incidence of scrotal skin cancer in chimney sweeps through exposure to coal-tar. The link between exposure to aromatic hydrocarbons and cancer was unequivocally proven in 1930.<sup>178,179</sup> Within the last few decades there has been a virtual explosion of scientific reports on aromatic hydrocarbons as carcinogenic agents that interfere with a multitude of biological processes.<sup>180</sup> Such investigations have contributed to the realization that aromatic hydrocarbons are among the main causes of human cancers.<sup>181</sup> The impetus drive for a better understanding of the biodegradation aromatic

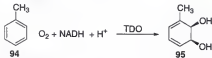
hydrocarbons biodegradation is therefore essential, since such understanding may allow development of bioremediation technology and production of chiral precursors for synthetic applications.

The degradation of aromatic hydrocarbons is carried out almost exclusively by dioxygenase-carrying microorganisms.<sup>182</sup> The initial step of the metabolic process is often the cycloaddition of oxygen to the aromatic moiety forming an unstable dioxetane<sup>183</sup> which is then enzymatically reduced to yield synthetically useful *cis*-glycols. This process constitutes the key step in the aromatic hydrocarbon degradation pathway and is a prerequisite for further fission and catabolism of the aromatic compounds, Scheme 2-18.



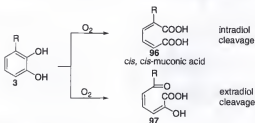
Scheme 2-18. Degradation of aromatics by microbial dioxygenases

Dioxygenases are involved in several stages of the aromatic hydrocarbon degradation pathway. Some dioxygenases, such as TDO, Scheme 2-19, are responsible for hydroxylation of the aromatic ring, while other dioxygenases, such as catechol



Scheme 2-19. Dihydroxylation of aromatic hydrocarbon by TDO

dioxygenases,<sup>184</sup> are known to be involved in the ring fission process of transforming catechol precursors into aliphatic products, as illustrated in Scheme 2-20. Generally, the catechol dioxygenases require no cofactor and can cleave the catechol moiety via either an intradiol or extradiol fashion, as depicted in Scheme 2-20. The intradiol-cleaving enzymes utilize Fe(III) to give metabolite **96**, while the extradiol-cleaving enzymes utilize Fe(II)<sup>185,186</sup> and in a few cases, Mn(II)<sup>187-189</sup> to give metabolite **97**.



Scheme 2-20. Modes of catechol cleavage

There are diverse classes of dioxygenases existing in nature that are capable of oxidizing a variety of organic substrates. However, the breakdown of the aromatic nucleus has received the most attention. Numerous studies have been launched to probe for a better understanding of this important metabolic process. As a result, there is a great deal of knowledge known about dioxygenases. They are multicomponent enzyme systems involving several non-heme iron proteins and require NADH for function.<sup>184,190-192</sup> To date there are a number of microorganisms that have been found to be capable of utilizing a wide range of carbon sources for energy and growth and these enzymes have been classified according to the type of substrate they oxidize. The following are some of the most common dioxygenases found to date: benzene dioxygenase (BDO), toluene dioxygenase (TDO), naphthalene dioxygenase (NDO), benzoate dioxygenase (BZDO), and biphenyl dioxygenase (BPDO). The overlapping in substrate specificity among the

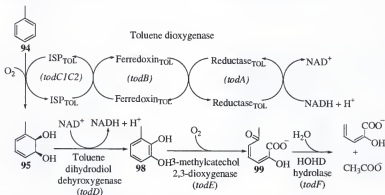
enzymes is common and therefore it is difficult to provide definitive information on the most appropriate type of dioxygenase for a particular type of biotransformation. In general terms, however, the TDO system is most suitable for *cis*-dihydroxylation of substituted benzene substrates and bicyclic arenes, NDO is particularly useful for bi- and tri-cyclic arenes, BPDO is more appropriate for larger polycyclic aromatic hydrocarbons and BZDO is required for benzoic acid substrates. More than twelve types of multicomponent ring-hydroxylating dioxygenases have been identified and purified.<sup>193</sup>

A number of mutant dioxygenases also have been identified and characterized and these have been instrumental in providing *cis*-dihydrodiols chiral synthons for numerous synthetic applications. For example, *Pseudomonas putida* mutant strain F39D<sup>190,194</sup> and UV4<sup>31</sup> provide a source of TDO, while JT103,<sup>195</sup> B13,<sup>196</sup> and BG1<sup>197</sup> are a source of BZDO and 9816/11<sup>192</sup> has been identified as the mutant strain for NDO. A mutant strain of a *Beijerinckia* species, *Spingomonas yanoikuyae* B8/36<sup>198</sup> has been identified as the source of BPDO and *Alcaligenes eutrophus* 335/B9 for BZDO.<sup>199</sup> Since, these mutant strains carry an ineffective *cis*-dihydrodiol dehydrogenase gene, the aromatic hydrocarbon metabolic pathways is terminated after the initial *cis*-dihydrodioxylation process. As a result, the *cis*-dihydrodiol metabolite accumulates. The genes encoding the TDO components in both F39D and UV4 strains have been cloned and expressed in *Escherichia coli* (pDTG601)<sup>200</sup> and (pKST 11),<sup>201</sup> respectively. The expression of these dioxygenases in *E. coli* hosts has been extremely useful in facilitating the production of chiral synthons for synthetic applications.

#### ***Pseudomonas putida* F39D**

In 1968 after a series of studies on microbial oxidation of aromatic hydrocarbons by soil bacteria, Gibson and colleagues discovered a strain of the bacteria, *Pseudomonas*

*putida* F1 (*PpF1*)<sup>194</sup> that could utilize benzene, toluene, and ethylbenzene as sources of carbon for energy and growth. Subsequent studies showed that the organism would not grow if the aromatic hydrocarbons were added directly to the growth media. However, good growth was observed when the substrate was added as a vapor. Toluene appeared to be the best substrate for cell growth. Cells that were induced with toluene were found to be able to metabolize a variety of other substrates. Isopropylbenzene was oxidized at approximately half the rate observed with toluene. Propylbenzene and butylbenzene were metabolized even slower. This evidence led investigators to believe that the bulkiness of the substituent on the aromatic ring has an inverse effect on the metabolic rate of the substrates. Oxygen uptake experiments<sup>202</sup> indicated that the organism consumed 1.0 mole of oxygen per mole of substrate and the oxidation process was initiated by incorporating both atoms of molecular oxygen into the aromatic nucleus to form (+)-*cis*-(1*S*,2*R*)-dihydroxy-3-methylcyclohexa-3,5-diene.<sup>203,204</sup> This metabolic transformation catalyzed by a multicomponent mononuclear non-heme iron



Scheme 2-21. Catabolic pathway for toluene used by *Pseudomonas putida* F1. Genes designations for individual proteins are shown in parentheses

oxygenase system, designated toluene dioxygenase (TDO).<sup>205</sup> The individual components of toluene dioxygenase have been purified and are organized as shown in Scheme 2-21.

The enzyme is believed to go through the following sequence of actions during the catabolism of aromatic substrate. Electrons are initially accepted from NADH by a flavoprotein, reductase<sub>TOL</sub>.<sup>206</sup> and transferred them to a small iron-sulfur protein, ferredoxin<sub>TOL</sub>.<sup>207</sup> Ferredoxin<sub>TOL</sub> then reduces the terminal dioxygenase, which is a large iron-sulfur protein that has been designated ISP<sub>TOL</sub>.<sup>208</sup> The reduced oxygenase catalyzes the addition of both atoms of molecular oxygen to toluene to form *cis*-toluene dihydrodiol. The further metabolism of *cis*-toluene dihydrodiol involves NAD<sup>+</sup>-dependent dehydrogenation reaction mediated by *cis*-toluene dihydrodiol dehydrogenase to form 3-methylcatechol.<sup>209</sup> Extradiol cleavage at the 2,3 position by 3-methylcatechol 2,3-dioxygenase yields 2-hydroxy-6-oxo-2,4 -heptadienoate, which is further metabolized to 2-hydroxypenta-2,4-dienoate and acetate.

The enzyme system of toluene dioxygenase has been extensively studied since its first disclosure in 1968. Individual enzymes within the metabolic pathway depicted in Scheme 2-21 have been purified and characterized.<sup>206</sup> Mutant *P. putida* with defective in each of the bacteria genes also have been identified and isolated.<sup>210,211</sup> A mutation that occurred at the *todD* gene resulted in a mutant strain *P. putida* 39/D. This mutant strain would no longer grow on toluene or benzene as the sole source of carbon. When *P. putida* 39/D was grown on glucose, in the presence of benzene, *cis*-benzenediol accumulated in the culture medium suggesting that the organism no longer carried a fully functional enzyme system as disclosed in Scheme 2-21. It appears that the toluene dihydrodiol dehydrogenase (*todD*) gene was disabled so that the metabolic pathway of

the *P. putida* 39/D is terminated at the *cis*-dienediol stage, Scheme 1-1. The early termination in this metabolic pathway makes this organism a valuable tool for the generation of synthetically useful chiral synthons.

#### ***Escherichia coli* JM109 (pDTG601)**

In order to study the individual enzymes, namely toluene dioxygenase, toluene dihydrodiol dehydrogenase, and 3-methylcatechol 2,3-dioxygenase, of the *P. putida* F1's metabolic pathway in detail, Gibson and colleagues constructed three clones that over-expressed the first three enzymes of the metabolic pathway shown in Scheme 2-21. The genes *todC1C2BA* that encode for toluene dioxygenase (reductase<sub>TOL</sub>, ferredoxin<sub>TOL</sub>, and ISP<sub>TOL</sub>) was cloned into a plasmid designated as pDTG601 and the expression of the genes was placed under control by a *tac* promoter which can be chemically induced by isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG). The plasmid was then placed in an *E. coli* host named JM109 (pDTG601).<sup>200</sup> The development of this recombinant microorganism has many advantages over the *P. putida* F39D strain in generating chiral metabolites for synthetic applications. Since handling and growing JM109 (pDTG601) is much easier than handling and growing *P. putida* F39D, JM109 (pDTG601) has enabled toluene dioxygenase to become much more accessible for scientific research. Large amount of JM109 (pDTG601) can be grown easily through fermentation processes and induced to over-express TDO for used in manufacturing of chiral synthons for various synthetic applications. Today the use of JM109 (pDTG601) has become routine and is an integral part of many synthetic ventures.

#### **Toluene Dioxygenase Substrate Specificity**

The broad substrate specificity of toluene dioxygenase has allowed for the generation of a diverse group of metabolites with varying degrees of substitutions.

Several key features of TDO-catalyzed *cis*-dihydroxylation processes have been elucidated, which have helped shed light into the nature of the active sites of toluene dioxygenase enzyme system.

For example, substrates with charged or very polar substituents, such as, hydroxy, amino, carboxy, nitro, sulfonic acid and sulfone, directly linked to the benzene ring have been found to be resistant to TDO dihydroxylation. In spite of these limitations, the dioxygenase enzymes are capable of accepting arene substrates with a wide range of substituents.

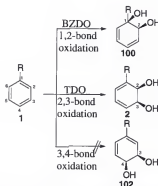
The degree of substitution and types of substituents on the aromatic rings also are key components in the TDO's catabolism processes of aromatic substrates. The likelihood a substrate is accepted by TDO decreases as the degree of substitution increases. When multiple electron-withdrawing substituents such as halogens are present, they reduce electron density from the aromatic ring thus hindering the activity of the aromatic moiety. On the other hand, when electron-donating groups are present, the reactivity of the substrates are increased; however, electron rich substituents tend to destabilize the *cis*-dihydrodiol metabolite and in many cases metabolites containing such substituents have been found to decompose spontaneously during the biotransformation or purification procedure. For the disubstituted substrates, a 1,3-arrangement of substituents suffer the so-called '*meta* effect' that would result in an increased inhibitory effect on the ring dihydroxylation rate in comparison with the substrates with substituents at the 1,2 or 1,4 positions.

The size of substituents on the aromatic ring also plays a role in determining the ability of TDO to accept the substrates. The smaller the substituent on the arene, the

better the substrate it is for the enzyme. For example, in the *cis*-dihydrodiols of alkylbenzenes, the rate of metabolism for substrate with a methyl substituent was observed to be twice as fast as for a substrate with *n*-butyl-substituent. The catabolism rate for *t*-butylbenzene was even slower and the *cis*-dihydrodiol metabolites of higher homologues are unknown.<sup>194</sup> Both naphthalene and biphenyl are good substrates for TDO enzymes, perhaps indicating that flat, rigid molecules fit better into the enzyme's active site than molecules with bulky, flexible, substituents.

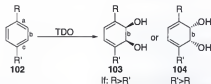
### Toluene Dioxygenase Regioselectivity

The TDO-catalyzed *cis*-dihydroxylation of monosubstituted aromatic substrates is very regioselective. With few exceptions of monohydroxylation, sulfoxidation, dealkylation and desaturation of substituents, the *cis*-dihydroxylation of monosubstituted benzenes by TDO occurred primarily at the benzene 2,3-bond. The hydroxylation at the 1,2-bond is the sole example of benzoic acid oxidation catalyzed by BZDO and no examples of dioxygenase-catalyzed *cis*-dihydroxylation at a 3,4-bond have been found (Scheme 2-22).



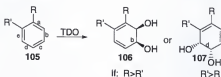
Scheme 2-22. Regioselectivity of dioxygenases in hydroxylation of aromatic substrates

Regioselectivity of TDO-catalyzed *cis*-dihydroxylation of disubstituted benzene substrates is restricted to unsubstituted arene bonds. For para-disubstituted benzene substrates of type **102**, the oxidation of the aromatic moiety will occur at bond **b** to afford *cis*-dienediol **103** if  $R > R'$  and give diol **104** if  $R' > R$ , (Scheme 2-23).



Scheme 2-23. Regioselectivity of TDO in biooxidation of 1,4-disubstituted aromatic substrates

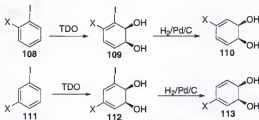
For ortho-disubstituted benzene substrates, Scheme 2-24, TDO-catalyzed oxidation will occur at either bonds **b** or **d** depending upon the size of the substituents on the ring. With a preference being shown for **b** hydroxylation if substituent  $R$  was larger than  $R'$  the oxidation will occur primary at bond **b** giving rise to metabolite  $\alpha$ -hydroxylated metabolite. On the other hand, if  $R'$  was larger than  $R$ , oxidation at bond **d** will be preferred.<sup>212,213</sup> When both substituents are of similar size, a mixture of *cis*-dihydrodiols formed due to oxidation at both bonds **b** and **d**.



Scheme 2-24. Regioselectivity of TDO in biooxidation of 1,2-disubstituted substrates

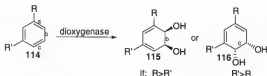
The directing effect of the substituents has practical implications and has been exploited for the preparation of *cis*-dienediols of type **110** and **113** (Scheme 2-25) that are not obtainable through conventional biotransformation. *Ortho*- or *meta*-substituted

iodobenzenes can be oxidized to the corresponding enantiopure *cis*-dihydrodiols and the metabolites was then subjected to catalytic hydrogenation would give rise to a new range of 3,4-*cis*-dihydrodiols unattainable by direct enzyme-catalyzed asymmetric dihydroxylation, (Scheme 2-25).<sup>213</sup>



Scheme 2-25. Chemoenzymatic approach to 3,4-*cis*-dihydrodiols

Regioselectivity for meta-disubstituted benzenes in TDO-catalyzed biotransformation is similar to that for ortho-disubstituted benzene substrates. There is a preference for attack at bonds **b** if  $R > R'$  to afford *cis*-dienediol type **115**. On the other hands, dihydroxylation of bond **c** will be predominant if  $R' > R$  to afford metabolite **116** as the major product (Scheme 2-26).



Scheme 2-26. Regioselectivity of 1,3-disubstituted aromatic substrates

Dihydroxylation of tri-substituted benzene substrates by TDO is rare and follows a pattern of regioselectivity similar to that observed in the oxidation of di-substituted benzene substrates by TDO. The broad substrate specificity of TDO allows for hydroxylation of bicyclic arenes, such as naphthalene and heteroarenes.<sup>183,214</sup>

Dihydroxylation of polycyclic arene of higher homologues of naphthalene also occur but

are carried out primarily by dioxygenases such as NDO and BPDO<sup>215,216</sup> that are capable of accommodating larger substrates. A strong preference for dihydroxylation at the bay-region bond is observed with a minor proportion of oxidation occurring at non-K positions (Figure 2-3). It is noteworthy that little evidence has been obtained for attack at the region of highest electron density in the angular arenes, *i.e.* the K-region, with either the NDO or BPDO enzymes.

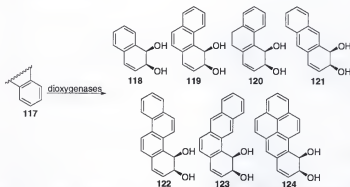


Figure 2-3. Preferred regioselectivity of mono- and polycyclic arene cis-dihydrodiols

The presence of one or more heteroatoms in the arene ring has been found to exert a strong influence on the regioselectivity of dioxygenase-catalyzed dihydroxylation. For bicyclic heteroarenes quinoline,<sup>217</sup> isoquinoline,<sup>217</sup> quinoxaline,<sup>217</sup> quinazoline,<sup>217</sup> benzothiophene,<sup>218</sup> 2-methylbenzo-thiophene,<sup>219</sup> benzofuran,<sup>220</sup> and 2-methylbenzofuran,<sup>219,220</sup> the *cis*-dihydroxylation of the carbocyclic arene ring is generally preferred over the heterocyclic ring. In some cases, mixture of metabolites have been observed as a result of oxidation at both carbocyclic and heterocyclic rings as in the case of the benzothiophene and benzofuran, Table 2-7.<sup>218,219</sup> However, where no carbocyclic

alternative exists, dihydroxylation occurs at the heteroarene ring itself, as in the case of thiophene and 3-methylthiophene.<sup>219</sup>

Table 2-7. TDO-catalyzed biotransformation of substrate containing heterocyclic ring system.

| Substrates                                                                                 | Metabolites                                                                         |                                                                                   |
|--------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| <br>125   |    |  |
| <br>128   |    |  |
| <br>131   |    |                                                                                   |
| <br>133   |    |                                                                                   |
| <br>135   |    |  |
| <br>138   |                                                                                     |                                                                                   |
| <br>139   |    |  |
| <br>142  |   |                                                                                   |
| <br>144 |  |                                                                                   |

Electron rich functional groups such as cyano-, vinyl-, alkoxy- and alkylthio-, appear to have a relatively strong directing effect. However, predictions of

regioselectivity of multi-substituted substrates are more difficult because of (1) uncertainty about the effective size of substituents in different conformations, (2) product instability and (3) further dioxygenase-catalyzed oxidation of the substituent.

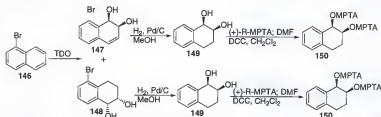
### **Toluene Dioxygenase Stereoselectivity**

The ability of toluene dioxygenase to oxidize a wide range of aromatic substrates while maintaining a high degree of optical purity is a remarkable biological process. The combination of such properties has no doubt made TDO an attractive catalyst for the production of synthetically useful chiral synthons. Indeed, the practicality of TDO in organic synthesis continues to fuel much of the interest within the chemoenzymatic synthesis arena. Today, many research groups from across the globe continue to devote their time and energy to gain better understanding of the enzyme's properties as well as its synthetic potential. Thanks to their efforts, more than three hundred *cis*-dihydrodiol metabolites of arenes have been reported and much information about the properties of the enzyme are known.

As the use of *cis*-dienediols for synthesis becoming more routine, the search for general methods for determining the optical purity and absolute stereochemistry of new metabolites is underway.<sup>221,222</sup> Traditional methods employed for such purpose have included the synthesis of these metabolites,<sup>220</sup> their chemical transformation to known compounds, conversion of the metabolites to diastereomers and derivatives,<sup>204</sup> X-ray crystallography,<sup>223</sup> stereochemical correlation<sup>5,6,8</sup> circular dichroism,<sup>224</sup> and NMR spectroscopy.<sup>221,222,225</sup>

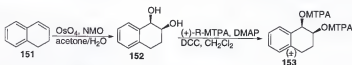
For example, the use of Mosher's chiral acids, R- or S- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenylacetic acid (MTPA), to form NMR-distinguishable diastereoisomeric esters in the presence of homochiral lanthanide shift reagent such as,

tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] europium (III),  $\text{Eu}(\text{hfc})_3$ ), has been useful in the determination of optical purity and absolute configuration of new metabolites. For instance, this approach has been used in determining the optical purity of metabolites obtained from biooxidation of 1-bromonaphthalene and 2-bromonaphthalene. In this effort, metabolites **147** and **148**, obtained from the biooxidation of corresponding 1-bromonaphthalene, were separated and converted into bis-MPTA protected ester **150** via catalytic hydrogenation and acylation of the *cis*-hydroxyl groups with MPTA (Scheme 2-27).



Scheme 2-27. Biotransformation of 2-bromonaphthalene

$^1\text{H}$ -NMR analysis of **150** derived from **147** and **148** in presence of an equal molar quantity of  $\text{Eu}(\text{hfc})_3$  showed a single signal for benzylic methane at  $\delta = 6.70$  ppm that matched with the standard sample of (-)-*cis*-tetrahydronaphthalene diol obtained from hydrogenation of the product of microbial dihydroxylation of naphthalene. By contrast, analysis of the  $^1\text{H}$ -NMR of the racemic Mosher esters **153** obtained from chemical



Scheme 2-28. Preparation of Mosher ester **153**

manipulation of 1,2-dihydronaphthalene **151**, Scheme 2-28, showed well-resolved signals for benzylic methines ( $\delta_{\alpha\text{-isomer}} = 6.85$  ppm and  $\beta\text{-isomer} = 6.70$  ppm) of both ester isomers. The combination of methods used above led to the establishment for enantiomeric excess of diol **147** and **148** to be >98%.

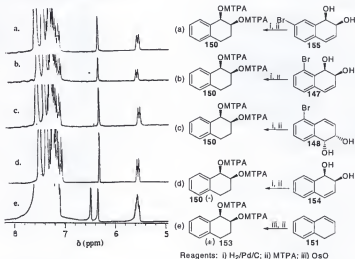
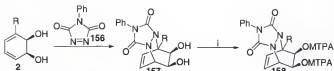


Figure 2-4. The use of Mosher's acid, MTPA, to determine optical purity of 1-bromonaphthalene diol.

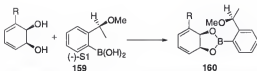
The formation of *syn*-cycloadducts between the *cis*-dihydrodiol metabolite of type 2 and 4-phenyl-1,2,4-triazoline-3,5-dione **156**, followed by protection of the free diols to form corresponding di-MTPA esters **158** provided another general method for enantiopurity determination  $^1\text{H}$ -NMR analysis (Scheme 2-29). The advantages of this method include: (1) the formation of crystalline products, which allow absolute configurations to be confirmed by X-ray crystallography, (2) good resolution of signals using  $^{19}\text{F}$  NMR analysis and (3) ready availability of both *R* and *S*-enantiomers of MTPA.



Reagents: i) (R) or (S)-x-methoxy-x-(trifluoromethyl)phenylacetyl chloride.

Scheme 2-29. Preparation of di-MTPA esters **158** for determination of *cis*-dienediol **2** absolute stereochemistry

More recently enantiopurity of *cis*-dihydrodiols can be determined using R- and S-2-(1-methoxyethyl)benzeneboronic acid (MPB) **159** followed by  $^1\text{H}$  NMR analysis (Scheme 2-30).<sup>221</sup>



Scheme 2-30. Preparation of boronic ester **160** for determination of *cis*-dienediol absolute stereochemistry

The attractive features of this method include the smaller quantity of *cis*-dihydrodiols required and the rapid rate of reaction, which allows less stable metabolites to be analyzed under milder conditions. The most sensitive and direct method for enantiopurity determination of *cis*-dihydrodiols involves the use of chiral stationary phase-high pressure liquid chromatography (CSP-HPLC). However, this method requires both enantiomers to be available in order to demonstrate their separation. Since the majority of *cis*-dihydrodiol metabolites are rather difficult to obtain in racemic form and appear to be enantiomerically homogeneous, the CSP-HPLC analysis method<sup>212</sup> is generally used in a complementary manner with the other methods.<sup>221,222</sup>

In term of stereoselectivity, it is now commonly accepted that biotransformation of arenes using TDO produces a single enantiomer. The oxidation of monosubstituted

benzenes by TDO, with the exception of fluorobenzene, gives almost exclusively a single enantiomer of the absolute configuration and with an enantiomeric excess of greater than



Figure 2-5. The preferred absolute stereochemistry of metabolites obtained from biotransformation of mono-substituted aromatic substrates

98% (Figure 2-5). The size of substituents on the aromatic ring plays a key role in defining the absolute stereochemistry of the metabolites. The bulkier the substituent, the greater directing effect it has on the stereochemical outcome of the oxidation process. When the size of the substituent is too small, as in the case of fluorobenzene, low stereocontrol was observed with enantiomeric excess ranging from 60–80%. This phenomenon is probably due to the relatively small size of the fluorine atom, which would allow more flexibility of the substrate within the active site of the TDO enzyme. Table 2-8<sup>226</sup> provides a few examples of TDO-catalyzed biotransformations of mono-substituted benzene with excellent enantiomeric excess.

Table 2-8. Preferred absolute configuration of cis-dihydrodiol metabolites of monosubstituted benzenes

| Metabolites | R                                                 | % ee |
|-------------|---------------------------------------------------|------|
| 98          | CH <sub>3</sub>                                   | >98  |
| 161         | Br                                                | >98  |
| 162         | CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> | >98  |
| 164         | CH=CH <sub>2</sub>                                | >98  |
| 165         | CH <sub>2</sub> OCH <sub>3</sub>                  | >98  |

For *ortho*-disubstituted benzene substrates, the bulkiest substituent on the ring exerts the strongest influence on the hydroxylation site and therefore controls the

stereochemistry of the metabolites. With the exception of *ortho*-difluorobenzene substrate,<sup>227</sup> the *cis*-dihydrodiol metabolites obtained from biotransformation of *ortho*-disubstituted benzenes appear to be enantiopure. The lower ee values obtained for the *cis*-dihydrodiol metabolites of *ortho*-difluoro-benzene (64%) again may be a result of the reduced steric requirements of the fluorine atoms (Table 2-9).<sup>226</sup>

Table 2-9. *cis*-Dihydrodiols from *ortho*-substituted benzenes

|  |                    |    |      |
|-----------------------------------------------------------------------------------|--------------------|----|------|
| Metabolites                                                                       | R                  | R' | % ee |
| 166                                                                               | F                  | F  | 64   |
| 167                                                                               | Me                 | F  | >98  |
| 168                                                                               | Br                 | F  | >98  |
| 169                                                                               | CH=CH <sub>2</sub> | Cl | >98  |
| 170                                                                               | I                  | Cl | >98  |

For meta-disubstituted benzene substrates, similar phenomenon of regio- and stereoselectivity was observed. Again, with the exception of *ortho*-difluorobenzene,<sup>227</sup> which afforded an ee of 64%, the enantiopurity of *cis*-dihydrodiol metabolites obtained from TDO-catalyzed oxidation processes are almost always greater than 98%. Table 2-10, lists a few examples of TDO-catalyzed biooxidation of meta-substituted benzene.<sup>226</sup>

Table 2-10. *cis*-Dihydrodiols from meta-substituted benzenes

|  |                 |    |      |
|-------------------------------------------------------------------------------------|-----------------|----|------|
| Metabolites                                                                         | R               | R' | % ee |
| 171                                                                                 | F               | F  | 56   |
| 172                                                                                 | I               | Br | >98  |
| 173                                                                                 | CF <sub>3</sub> | F  | >98  |
| 174                                                                                 | I               | F  | >98  |
| 175                                                                                 | Me              | F  | >98  |

Dioxygenase-catalyzed *cis*-dihydroxylation of para-disubstituted benzene substrates, on the other hand, produces *cis*-dihydrodiols with a wide range of enantiopurity.<sup>212</sup> The relative sizes of the substituents ( $\text{CF}_3 > \text{I} > \text{Br} > \text{Cl} > \text{Me} > \text{F} > \text{H}$ ) provide an empirical method for both the prediction of regioselectivity and facial selectivity during TDO-catalyzed *cis*-dihydroxylation of benzene substrates. The general structure depicted in Table 2-11<sup>226</sup> indicates that the preference for a particular enantiomer will be related to the differential in size between the large R and small R' groups.

Table 2-11. *cis*-Dihydrodiols from para-substituted benzenes

|           |               | If: $R > R'$ |      |
|-----------|---------------|--------------|------|
| Compounds | R             | R'           | % ee |
| 176       | I             | F            | 88   |
| 177       | I             | Me           | 80   |
| 178       | Br            | Me           | 37   |
| 179       | Cl            | Me           | 15   |
| 180       | Br            | F            | 56   |
| 181       | I             | F            | 88   |
| 182       | $\text{CF}_3$ | I            | >98  |
| 183       | $\text{CF}_3$ | Me           | >98  |

groups. The absolute configuration of the preferred *cis*-dihydrodiol enantiomer from TDO-catalyzed oxidation for di-substituted benzene substrates is summarized in Figure 2-6.<sup>227</sup>

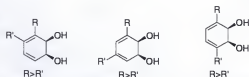


Figure 2-6. Preferred absolute configuration of *cis*-dihydrodiol metabolites of disubstituted benzenes

TDO also is capable of oxidizing a wide range of polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic hydrocarbons such quinoline, isoquinoline, quinoxaline, with good yields and excellent enantiomeric excess.<sup>217</sup> Table 2-12<sup>226</sup> lists some of these metabolites to illustrate the structural diversity obtained from this transformation.

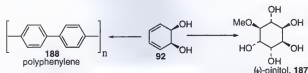
Table 2-12. *cis*-dihydrodiols from polycyclic aromatic hydrocarbons

| Substrates                                                                          | Products                                                                           | ee% |
|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-----|
|    |   | >98 |
|                                                                                     |   | >98 |
|    |   | >98 |
|    |   | >98 |
|    |   | >98 |
|  |  | >98 |

### Synthetic Applications of Dihydrodiols

The synthetic utility of the new chiral centers derived from the *cis*-dihydroxylation of arene via TDO has been extensively reviewed. Despite the important original

disclosure made by Gibson on aromatic degradation by *P. putida* in 1968, it took almost 20 years for the synthetic application of this discovery to be realized with the syntheses of racemic pinitol by Ley<sup>30</sup> in 1987 and polyphenylene<sup>31,32</sup> by ICI (Scheme 2-31).



Scheme 2-31. Historically important application of arene *cis*-diols in synthesis

Research groups around the world soon followed these initiatives and demonstrated the diversity of substrate range of the dioxygenase enzymes. To date, several hundred *cis*-diols that have been isolated and identified, and new metabolites continue to be discovered that could eventually lead synthetic chemists toward new applications and more diverse targets. The rich functionality of the metabolites offers flexibility for chemical manipulations including: asymmetric Diels-Alder reactions, epoxidation, photochemical oxygenation, metallation, diol cleavage, diene cleavage, carbene additions and ozonolysis that are synthetically useful (Figure 2-7).

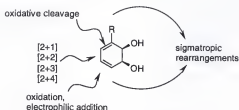


Figure 2-7. Local bond-forming sites in the *cis*-dienediols

Such chemical manipulations have been extremely rewarding in the creation of a whole new class of "primary synthons" that possess even greater versatility and applicability than the original metabolites for synthesis of complex synthetic targets

(Figure 2-8).<sup>24</sup> Such “primary synthons” can be further manipulated into “secondary synthons” (Figure 2-9) for synthetic applications that have proved very fruitful.<sup>24</sup> In this

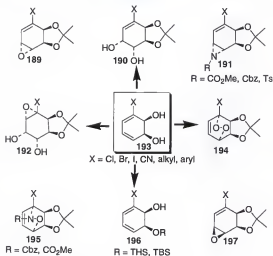


Figure 2-8. Examples of primary synthons

manner, cyclohexanoids such as (+)-pinitol<sup>228</sup> and D-*myo*-inositol derivatives<sup>229</sup> have been prepared by making use of the possibility of functionalizing every carbon atom of the glycol in a stereo-control approach. Cyclopentanoid synthons on the other hand have been used for the synthesis of prostaglandins and terpenes via ring-opening/closure sequence.<sup>33</sup> Rare carbohydrates such as D- and L- erythrose<sup>230</sup> and L-ribonolactone<sup>231</sup> were obtained from chlorobenzene as were pyrrolizidine alkaloids.<sup>232</sup> In addition, other complex natural products such as (-)-zeylena, (+)-lycoricidine, (-)-specionin, and (+)-pancratistatin also have been synthesized efficiently using *cis*-dienediols.

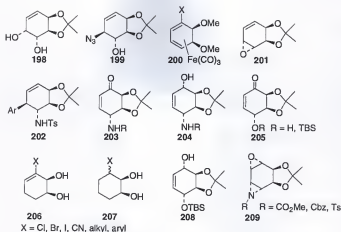


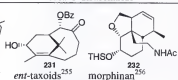
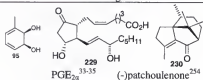
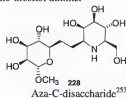
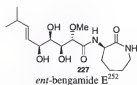
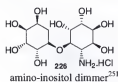
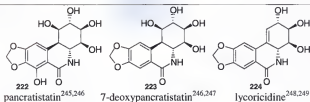
Figure 2-9. Example of secondary synthons

Hudlicky and his research group have been pioneers in this effort. Most of the work of the group to date has been conducted with a diverse range of diols derived from the biotransformation of corresponding arenes by TDO.<sup>25</sup> Table 2-13 lists some representative synthetic products that are illustrative of the applicability and versatility of TDO as a synthetic tool.

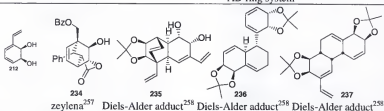
The synthetic potential of dioxygenase enzyme system, such as TDO, provides a viable research tool that currently is used by a growing number of research groups across the globe for a variety of synthetic applications, and the activity within the field continues to grow.

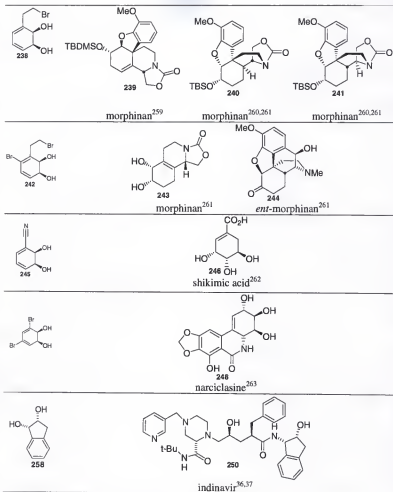
Table 2-13. The use of *cis*-dienediols in synthesis

| Diols                                    | Product of asymmetric syntheses    |                                                       |                                                           |
|------------------------------------------|------------------------------------|-------------------------------------------------------|-----------------------------------------------------------|
|                                          |                                    |                                                       |                                                           |
| 211                                      | 212                                | 213                                                   | 214                                                       |
| conduritol C <sup>233</sup>              | conduramine A-1 <sup>234,235</sup> | D-chiro-inositol <sup>236</sup>                       | (-)-pinitol <sup>237</sup>                                |
|                                          |                                    |                                                       |                                                           |
| 215                                      | 217                                | 216                                                   |                                                           |
| deuterated hexose <sup>234,235,238</sup> | L-ascorbic acid <sup>239</sup>     | hexoses, <sup>240</sup><br>azahexoses, <sup>240</sup> | aminohexoses, <sup>240</sup><br>carbasugar <sup>240</sup> |
|                                          |                                    |                                                       |                                                           |
| 218                                      | 219                                | 220                                                   |                                                           |
| kifunensine <sup>241,242</sup>           | specionin acetate <sup>243</sup>   | (+)-aspicilin <sup>244</sup>                          |                                                           |

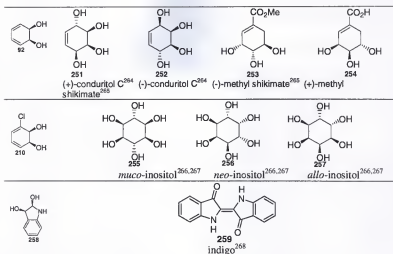


## AB-ring system





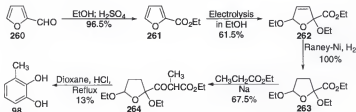
Diols      Products of non-asymmetric syntheses



### Substituted Catechols

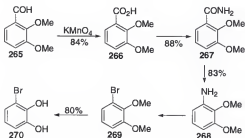
Catechol and its derivatives are common structural features of many natural products. Many of them are known to possess diverse biological activity, such as antihyperglycemic,<sup>269</sup> antimutagenic,<sup>270</sup> antibiotic, and cytotoxic activities.<sup>271</sup> For these reasons, the role of catechol derivatives has become a topic of increased study in recent years. However, despite their chemical and biological importance, only a limited number of studies have been devoted to the synthesis of catechols derivatives. Available synthetic methods for generation of substituted catechols tend to be cumbersome, non-selective and involve harsh conditions.<sup>272,273</sup> The following paragraphs provide a few examples of how substituted catechols are made through the use of conventional chemical methods. These examples give details that are illustrative of the difficulties associated with using currently available methods for the synthesis of such compounds.

The synthesis of toluene catechols, for instance, involves 7 steps with an overall yield of 5% starting from furfural<sup>274</sup> (Scheme 3-32).



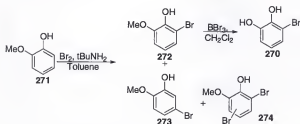
Scheme 2-32. Synthesis of methylcatechol from furfural

Preparation of bromocatechol **270** was achieved by first converting 2,3-dimethoxybenzaldehyde **265** to 2,3-dimethoxybenzoic acid **266** through permanganate oxidation.<sup>275</sup> The acid was then converted to 2,3-dimethoxybenzamide **267**, which was then subjected to a modified Hoffmann rearrangement process<sup>276</sup> to furnish 2,3-dimethoxyaniline **268**. The resulting aniline **268** was converted to 2,3-dimethoxybromobenzene **269**, using a modified Sandmeyer procedure.<sup>277</sup> Finally, demethylation of dimethoxybromobenzene **269** with aluminum trichloride in chlorobenzene<sup>278</sup> produced 3-bromocatechol **270** in 5 steps with an overall yield of 49% (Scheme 2-33).



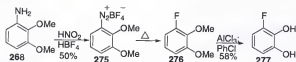
Scheme 2-33. Synthesis of bromocatechol

Bromination and demethylation of quaiacol is another approach to 3-bromocatechol. Bromination of quaiacol in a solution of *t*-BuNH<sub>2</sub> and toluene provided 2-bromo-6-methoxyphenol **272** as the major product plus a small amount of 3-bromo-6-methoxyphenol **273** as well as trace amount of polybrominated products **274**. The side-products were minimized when two or more molar equivalents of quaiacol were used to give the desired product in a 60% yield. Demethylation of 2-bromo-6-methoxyphenol **272** with boron tribromide in methylene chloride resulted in 3-bromocatechol,<sup>279</sup> as illustrated in Scheme 2-34.



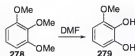
Scheme 2-34. Synthesis of bromocatechol from quaiacol.

In the synthesis of 3-fluorocatechol **277**, 2,3-dimethoxyaniline **268** was treated with nitric acid and quenched with floroboric acid to give diazonium salt **275**.<sup>280</sup> The salt was pyrolyzed to give 2,3-dimethoxyfluorobenzene **276**, which was subjected to demethylated by aluminum chloride to give 3-fluorocatechol **277** in a 29% overall yield (Scheme 2-35).<sup>281</sup>



Scheme 2-35. Synthesis of 3-fluorocatechol

The synthesis of anisole catechol **261** was accomplished via demethylation of aryl methyl ether with thioethoxide ion in dimethyl formamide to give an excellent yield of 96% (Scheme 2-36).<sup>282</sup>



Scheme 2-36. Synthesis of 3-methoxycatechol

The above examples are representative of chemical methods available for the syntheses of 1,2-monosubstituted catechols. Most methods involve the use of harsh conditions such as strong acids, or oxidants to cleave alkylated catechols with poor selectivity and low yields.

Enzymatic approaches to catechol synthesis are available but under-explored. To the best of our knowledge, only three or four methods of making catechols through enzymatic means exist, in addition to the original report by Gibson. The process by Frost<sup>283</sup> involves the use of *E. coli* AB2834/pKD136/pKD9.069A for conversion of glucose to the parent catechol. Another method involved the oxidation of substituted 2-nitrophenols to various catechols with nitrophenol oxygenase via *Pseudomonas* Sp. Strain T-12 to generate various substituted catechols.<sup>284</sup> Recently, Yoshida and co-workers reported a new organism capable of oxidizing aromatic substrates to catechols in one step.<sup>285</sup>

#### Catechol Dehydrogenases – *Escherichia coli* JM109 (pDTG602)

Studies of soil bacteria degradation of aromatic compounds led David Gibson in the late 1960's to elucidate the metabolic pathway as shown in Scheme 1-1. Although the use of TDO as a means of generating chiral synthons for synthetic applications is well

established, the use of the second enzyme, TDDH, has been limited to explorations in cloning.<sup>29</sup> This is surprising in view of the potential of simple catechols to serve as synthetic building blocks for various synthetic applications.

In 1989 the gene *todC1C2BAD* encodes for TDDH, which is responsible for the transformation of aromatics to corresponding catechols was cloned and expressed into an *E. coli* host JM109 (pDTG602).<sup>200</sup> Although the organism has been in existence for more than a decade, there are few reports of the use of the organism for production of catechols.

### Combretastatins

#### Isolation and Biological Activity

The importance of natural products in biomedical sciences is well established, as illustrated by the number of scientific publications devoted to this subject. New natural products are being discovered everyday and their biological affects are routinely being evaluated. Today, natural products and their derivatives are integral parts of every drug discovery program. As part of the effort in combating chronic diseases such as cancer, AIDS, and TB, the worldwide exploratory survey of plants for natural products with desirable biological activity is an active and promising field, offering hope for combating chronic diseases.

In this effort, George Pettit and co-workers have traveled to all corners of the world to explore new sources of natural products from medicinal native plants. In 1981, their efforts led them to investigate the medicinal potential of an African bush willow tree, *Combretum caffrum*, of the *Combretaceae* family.<sup>286</sup> The tree has been used by the natives for centuries for treatment of leprosy, cancer, and mental illness.<sup>287-289</sup> In 1985, a large-scale extraction of the bark of the African bush willow tree was initiated, yielding

0.7g of combretastatin A-1 ( $9.1 \times 10^{-4}$  %) and 39.6mg ( $5.1 \times 10^{-5}$  %) of combretastatin B-

1.<sup>290</sup> Structural analyses of combretastatin A-1 and B-1 via IR and UV suggested the presence of aromatic systems. High resolution EIMS indicated molecular formula  $C_{18}H_{20}O_6$  and  $C_{18}H_{22}O_6$  for combretastatin A-1 and B-1, respectively.  $^1H$ -NMR spectroscopic data of the natural products exhibited signals for four methoxy group protons and indicated that combretastatin B-1 is a dihydro derivative of A-1.  $^1H$ -NMR data of combretastatin A-1 showed four aromatic protons, two of which are magnetically identical and relatively shielded at  $\delta$  6.460. The other two are doublets of an AB spin system appearing at  $\delta$  6.310 and  $\delta$  6.691 with a coupling constant ( $J=8.64$  Hz) typical of two *ortho* coupled aromatic protons. Another set of AB spin system appears as doublets at  $\delta$  6.453 and 6.523 with coupling constant ( $J=12.2$  Hz) and two exchangeable protons at  $\delta$  5.438, suggesting the presence of dihydroxy functionality. The  $^1H$ -NMR data for combretastatin A-1 and B-1 is summarized in Table 2-14.

Table 2-14. Combretastatin A-1 and B-1  $^1H$ -NMR\*

| Combretastatin A-1   |               |                                  | Combretastatin B-1                 |             |                                |
|----------------------|---------------|----------------------------------|------------------------------------|-------------|--------------------------------|
| protons              | ppm           | Coupling Constant (Hz)           | protons                            | ppm         | Coupling (Hz)                  |
| 3,5-OCH <sub>3</sub> | 3.597 (6H, s) | $J_{AB}=8.64$<br>$J_{A'B'}=12.2$ | CH <sub>2</sub> -CH <sub>2</sub> - | 2.851(4H,m) | $J_{AB}=8.36$<br>$J_{BA}=8.36$ |
| 4-OCH <sub>3</sub>   | 3.760 (3H, s) |                                  | 4'-OCH <sub>3</sub>                | 3.827(3H,s) |                                |
| 4'-OCH <sub>3</sub>  | 3.770 (3H, s) |                                  | 3,5-OCH <sub>3</sub>               | 3.831(6H,s) |                                |
| 2',3'-OH             | 5.438 (2H, s) |                                  | 4-OCH <sub>3</sub>                 | 3.856(3H,s) |                                |
| 5'-H                 | 6.310 (1H, d) | $J_{B'A'}=12.2$<br>$J_{BA}=8.6$  | 2',3'-OH                           | 5.382(1H,s) |                                |
| CH=CH                | 6.453 (1H, d) |                                  |                                    | 5.398(1H,s) |                                |
| 2,6-H                | 6.460 (2H, s) |                                  | 5'-H                               | 6.390(1H,d) |                                |
| CH=CH                | 6.523 (1H, d) |                                  | 2,6-H                              | 6.420(2H,s) |                                |
| 6'-H                 | 6.691 (1H, d) |                                  | 6'-H                               | 6.577(1H,d) |                                |

\*chemical shift assignments relative to TMS in CDCl<sub>3</sub> solution. For proton-numbering – see Figure 2-11

Further NMR studies, including  $^{13}C$ -NMR and nuclear overhauser effect difference spectroscopy (NOEDS), of combretastatin A-1 and B-1, suggested structural features as

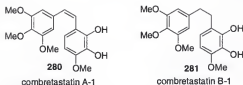


Figure 2-10. combretastatin A-1 and B1

shown in Figure 10.  $^{13}\text{C}$ -NMR data of combretastatin A-1 and B-1 are summarized in Table 2-15.

Table 2-15. Combretastatin A-1 and B-1  $^{13}\text{C}$ -NMR\*

| Carbon               | Combretastatin A-1 | Combretastatin B-1 |
|----------------------|--------------------|--------------------|
| 1                    | 132.49             | 138.18             |
| 2                    | 106.13             | 105.67             |
| 3                    | 152.80             | 153.05             |
| 4                    | 132.67             | 132.35             |
| 5                    | 152.80             | 153.05             |
| 6                    | 106.13             | 105.67             |
| 1a                   | 130.21             | 36.49              |
| 1'a                  | 124.06             | 31.82              |
| 1'                   | 117.91             | 121.55             |
| 2'                   | 141.72             | 142.19             |
| 3'                   | 137.42             | 136.21             |
| 4'                   | 146.37             | 145.40             |
| 5'                   | 102.98             | 102.52             |
| 6'                   | 120.17             | 120.32             |
| 3,5-OCH <sub>3</sub> | 55.85              | 56.12              |
| 4-OCH <sub>3</sub>   | 60.79              | 60.18              |
| 4'-OCH <sub>3</sub>  | 56.16              | 56.18              |

\*see Figure 2-11 for carbon-numbering

Combretastatin A-1's structure was subsequently and unequivocally established by X-ray crystallography, Figure 2-11.<sup>291</sup>

In numerous studies of the biological properties of combretastatins A-1 and B-1, the compounds were shown to cause mitotic arrest<sup>292</sup> in cultured cells. Competitive inhibition studies between combretastatins A-1 and B-1 and the well-known antimitotic agents podophyllotoxin **282**, steganancin **283**, colchicines **284**, suggested that

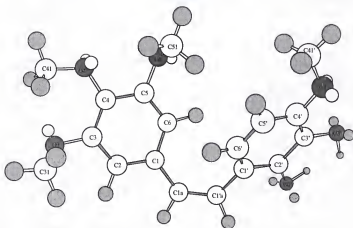
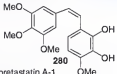
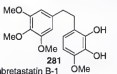
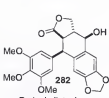
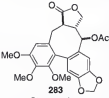
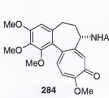


Figure 2-11. Crystal structure of combretastatin A-1

combretastatins share a common binding site on tubulin and are competitive inhibitors of colchicine, as summarized in Table 2-16, Experiment II.<sup>293-297</sup> Combretastatin A-1 was found to be capable of displacing 97.8% of colchicine from its binding site, which suggests it has an affinity for the active site on tubulin. These natural products also were found to be potent inhibitors of microtubule assembly at 2-3  $\mu\text{M}$  ( $\text{IC}_{50}$ ) concentrations, Table 2-16, Experiment I.

These biological properties demonstrated for combretastatin A-1 and B-1 encouraged further exploration and biological evaluation of this class of natural products. Since the disclosure of combretastatin A-1 and B-1, a series of combretastatins have been reported, Figure 2-12. The majority of the members of this class of compounds have been shown to be potent against a series of cancer cell lines. They interact with and disrupt the vascular life support system of tumors, and in the process, starve and prevent cancerous cells from metastasizing. The results of preclinical studies in which tumor

Table 2-16. Inhibition of microtubule assembly and binding of colchicine to tubulin by combretastatin A-1 and B-1

| <div style="display: flex; justify-content: space-around; align-items: flex-end;"> <div style="text-align: center;">  <p>Combretastatin A-1<br/>280</p> </div> <div style="text-align: center;">  <p>Combretastatin B-1<br/>281</p> </div> </div>                                                                                                                                           |                                               |                                    |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|------------------------------------|
| <div style="display: flex; justify-content: space-around; align-items: flex-end;"> <div style="text-align: center;">  <p>Podophyllotoxin<br/>282</p> </div> <div style="text-align: center;">  <p>Steganacin<br/>283</p> </div> <div style="text-align: center;">  <p>Colchicine<br/>284</p> </div> </div> |                                               |                                    |
| Drug                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Experiment I                                  | Experiment II                      |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Microtubule Assembly<br>ID <sub>50</sub> (μM) | Colchicine Binding<br>% of control |
| Combretastatin A-1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 2                                             | 2.2                                |
| Combretastatin B-1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 3                                             | 14                                 |
| Combretastatin                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 11                                            | 34                                 |
| Podophyllotoxin                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 3                                             | 13                                 |
| Steganacin                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 6                                             | 49                                 |
| Colchicine                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 6                                             | ---                                |

<sup>a</sup>Defined as the drug concentration inhibiting the extent of microtubule assembly by 50% (adapted from published work by Pettit et al.)<sup>291,298</sup>

cells were treated with combretastatins showed that these agents produced tumor shrinkage and ultimately tumor disappearance. Thus, this class of natural products may offer a new approach to cancer treatment that is a radical departure from conventional drugs.

Among the combretastatins known to date, (Figure 2-12), combretastatin A-4 has been identified as the most active vascular targeting and anti-angiogenesis agent.<sup>299</sup> In 1998, clinical trials with its water-soluble phosphate derivative<sup>300,301</sup> 291, Figure 2-13,

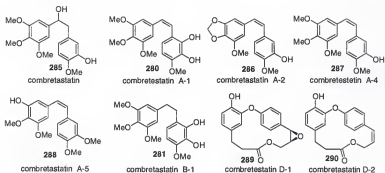


Figure 2-12. Structural variation in Combretastatins

began<sup>302</sup> and are continuing into phase II. Today studies continue to investigate the structure-activity relationship of substituted stilbene derivatives of this type.<sup>303-306</sup>

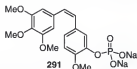


Figure 2-13. Water soluble phosphate derivative of combretastatin A-4

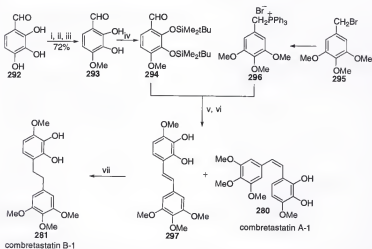
From these studies it has been shown that the 3,4,5-trimethoxy aryl unit, a small group on the 4'-position, and separation of two aryl rings by a two carbon unit, are important for antimitotic activity, while substitution with a hydroxyl group on the 3'-position is not essential.<sup>303,307-309</sup>

## Combretastatins Syntheses

### Combretastatin A-1 and B-1

Early syntheses of combretastatins and their analogues used the Wittig reaction as the key step for installing the biaryl moieties flanked by a methylene bridge. In the synthesis of combretastatins A-1 and B-1, 2,3,4-trihydroxybenzaldehyde **292** was treated with sodium borate-decahydrate (borax) in water for selective formation of the 2,3-borate

ester, which allowed for specific methylation of the 4-hydroxy group by dimethylsulfate. The reaction mixture was quenched with concentrated hydrochloric acid to afford dihydroxybenzaldehyde **293**,<sup>310,311</sup> which was then treated with diisopropylethylamine and *t*-butyldimethylsilyl chloride (TBDMSCl) in dimethylformamide (DMF) to form 2,3-*t*-butyldimethylsilyl ether **294**.<sup>312</sup> The synthesis of combretastatin A-1 was then accomplished by coupling TBDMS-protected aldehyde **294** with phosphonium salt **296**, (readily prepared via treating 3,4,5-trimethoxybenzyl bromide **295** with *t*-BuLi and followed with triphenylphosphine in THF) to give a 92.5% yield of a 9:1 ratio of *Z/E* combretastatin A-1 (Scheme 2-37). The products mixture was reduced to via hydrogenation to arrive at combretastatin B-1. The disadvantage of this approach to combretastatins is the resulting mixture of products requires separation.

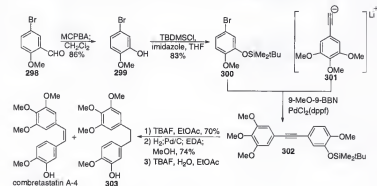


Conditions: i) Borax, NaOH; ii)  $(\text{MeO})_2\text{SO}_2$ ; iii) HCl; iv) DMF, EtN(iPr)<sub>2</sub>, TBDMS-Cl; v) BuLi, THF, -15°C-rt; vi) TBAF, THF; vii) H<sub>2</sub>/C/Pd, MeOH

Scheme 2-37. Total synthesis of combretastatins A-1 and B-1

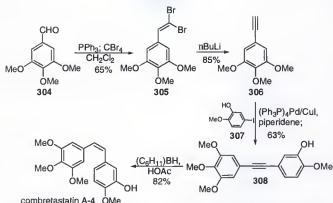
## Combretastatin A-4

Since combretastatin A-4 is the most potent anticancer agent among the combretastatins discovered to date, this drug and its derivatives have been the focus of many syntheses. As shown in the Scheme 2-38, arylacetylene **302**, which can be fashioned in a few steps, was the key intermediate in an approach developed by Furstner *et al.* to combretastatin A-4.<sup>298</sup> The key step in this approach involved the coupling of arylbromide **300** with the lithiated trimethoxyacetylene **301** via a 9-MeO-9-BBN-mediated Suzuki-type cross coupling reaction to produce internal alkyne **302**. Selective hydrogenation of **302** with a Lindlar's catalyst (5%Pd/C, ethylenediamine) in MeOH, followed by deprotection of the TBDMS-group with aqueous TBAF provided a 86:14 mixture of combretastatin A-4 and its saturated analogue **303** (Scheme 2-38).

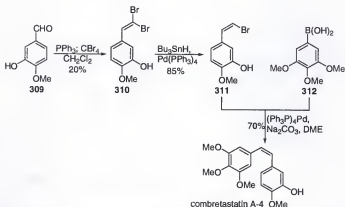


Scheme 2-38. Suzuki approach to combretastatin A-4

Using similar strategy, Lawrence and colleagues disclosed their approach to combretastatin A-4 with an overall yield of 29% over 5 steps (Scheme 2-39).<sup>313</sup> Again, the key step in the synthesis involved the coupling of the readily available trimethoxyacetylene **306**<sup>298</sup> with aryl iodine **307** to give diarylalkyne **308**. The internal



Scheme 2-39. Synthesis of combretastatin A-4 via Suzuki cross-coupling reaction  
alkyne was then sequentially treated with dicyclohexylborane and acetic acid to give combretastatin A-4 in 82% yield. This approach to combretastatin A-4 is much more straight forward, as it requires no protecting group and the alkene moiety flanking the aryl units is constructed with greater stereoselectivity.



Scheme 2-40. Synthesis of combretastatin A-4

More recently, Lawrence *et al.* disclosed another short synthesis of combretastatin A-4 utilizing Suzuki coupling reaction.<sup>314</sup> In this method, the coupling of the *cis*-vinylbromo styrene 311 with trimethoxyboronic acid 312 produced combretastatin A-4 in three steps and eliminated the necessary reduction step required in the earlier syntheses (Scheme 2-40).

### Combretastatin D-1 and D-2

Combretastatin D-1 and D-2 were isolated in trace amount from the South African folk medicine tree *Combretum caffrum*.<sup>315,316</sup> Structurally they differ from the A and B series in that they contain a 15-member biaryl ether macrolactone resembling that of the caffrane<sup>317</sup> ring system. These natural products and their derivatives, Figure 2-14, have

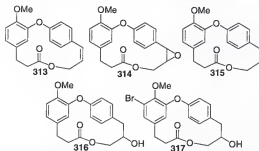


Figure 2-14. Combretastatin D derivatives

been shown to be active against the murine P-388 lymphocytic leukemia cell line.

Combretastatin D-1 shows a PS cell line activity corresponding to  $ED_{50}$  3.3  $\mu\text{g/mL}$  and D-2 activity corresponding to  $ED_{50}$  5.2  $\mu\text{g/mL}$ .<sup>316</sup> Structure-activity-relationships for combretastatin D-1 and D-2 and their derivatives on the tubulin polymerization process at various concentration have been determined<sup>318</sup> and the results are summarized in Table 2-17.

Table 2-17. Effect of tested compounds on tubulin polymerization.<sup>a</sup>

| Compound   | Degree of tubulin polymerization |        |        |        |
|------------|----------------------------------|--------|--------|--------|
|            | 0.25mM                           | 0.5 mM | 1.0 mM | 2.0 mM |
| Colchicine | 4%                               | 3%     | 4%     | 0%     |
| <b>313</b> | 16%                              | 19%    | 27%    | 35%    |
| <b>314</b> | 0%                               | 20%    | 27%    | 39%    |
| <b>315</b> | 5%                               | 28%    | 35%    | 54%    |
| <b>316</b> | 27%                              | 47%    | 62%    | 76%    |
| <b>317</b> | 3%                               | 21%    | 39%    | 67%    |

<sup>a</sup> assay with epothilone B<sup>319</sup> (20 $\mu$ M) resulted in 100% polymerization.

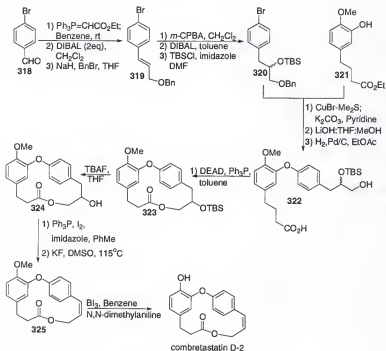
In contrast to colchicines and combretastatins A, which inhibit tubulin polymerization, combretastatin D derivatives, Figure 2-14, favor to various degrees the formation of microtubules. Derivative **316** seems to be most active (Table 2-17), capable of promoting 76% polymerization at concentration of 2.0 mM.

The structural simplicity of the combretastatin D series and their important biological activity mentioned above make them attractive lead for the development of new drugs. Couladouros and colleagues pioneer in this area, have developed efficient methods for total synthesis of combretastatin D-1 (Scheme 2-41),<sup>320</sup> and D-2 (Scheme 2-42).<sup>321</sup>

In an approach to combretastatin D-2, Wittig-type elongation of commercially available para-bromobenzaldehyde **318** by means of  $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$  afforded conjugated ester, which was subjected to diisobutyl aluminium hydride (DIBAL) reduction and the resulting allylic alcohol was protected as benzyl ether **319**. Epoxidation of **319** using *m*-CPBA (3-chloroperoxybenzoic acid) and regioselective ring opening with DIBAL<sup>322</sup> afforded, after silylation with TBSCl, the desired oxygenated synthon **320**. Ullman-type

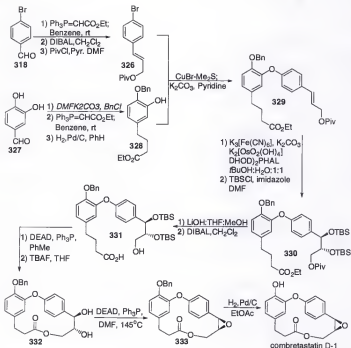
coupling of arylbromide **320** with phenol **321**,<sup>317</sup> followed by deprotection of the terminal carboxylic and hydroxyl functionalities afforded the saturated acid **322**.

Macrolactonization of **322** via a modified Mitsunobu-type condition<sup>323,324</sup> afforded **323** in 91 % yield. Subsequent desilylation, promoted by fluoride anions, afforded the alcohol **324** in 94 % yield. Dehydration of the alcohol **324** was achieved using Samuelson's



Scheme 2-41. Total synthesis of combretastatin D-2

conditions<sup>325</sup> by first replacing the hydroxyl group with iodine and subsequent dehydrohalogenation in refluxing DMF in the presence of excess KF afforded methyl combretastatin D-2 **325**. Combretastatin D-2 was finally prepared after demethylation of **325** in 17 % yield following Boger's procedure.<sup>317</sup>



Scheme 2-42. Total synthesis of combretastatin D-1

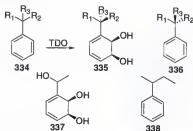
Similarly, the synthetic approach to combretastatin D-1 began with an Ullman-coupling of the monobenzylated catechol derivative 328, which was prepared from commercially available aldehyde 327 by selective benzylation, followed by Wittig-type carbon elongation and reduction of the resulting double bond, with aryl bromide 318. The biaryl ether 329 was subjected to asymmetric dihydroxylation by means of the well-known Sharpless catalyst (DHQD)<sub>2</sub> PHAL (hydroquinidine 1,4-phthalazinediyl diether)<sup>326</sup> followed by bis-silylation of the diol to arrive at the bis-silylether 330.

Subsequent hydrolysis of the ester functionality with LiOH and reductive cleavage of the pivaloate ester with DIBAL afforded acid **331**. Macrolactonization of this saturated disubstituted acid under the same protocol previously used for the synthesis of combretastatin D-1 furnished diol **332**, after desilylation of the TBS-groups with TBAF. The epoxide functionality of combretastatin D-1 was installed via the well-known phosphoranes and oxyphosphonium salts catalyzed diol cyclodehydration protocol,<sup>327,328</sup> by heating diol **332** with excess diethyl azodicarboxylate (DEAD) and  $\text{Ph}_3\text{P}$  at  $145^\circ\text{C}$  for 40 min followed by hydrogenolysis of the benzyl group to obtain combretastatin D-1 in high overall chemical yield of 83 % after crystallization.

### CHAPTER 3 RESULTS AND DISCUSSION

#### *Pseudomonas putida* F39D and *E. coli* JM109 (pDTG601) oxidation of 1-phenyl-1-ethanol

The utility of *cis*-dihydroxydihydrobenzene metabolites obtained from TDO-catalyzed *cis*-dihydroxylation of aromatic hydrocarbons in asymmetric synthesis is firmly established. Several recent reviews highlight the growing activity in this field<sup>24,26,226</sup> as investigations continue to probe for the enzyme's properties and functions.<sup>329-332</sup> To date, over three hundred homochiral diols have been identified from the oxidations of simple, fused, and biphenyl-type aromatics.<sup>24,26,226</sup> Despite this solid endeavor in the field, very few studies have been designed to study the enzyme's capacity in resolving substrates with remote chiral center of type 334 (Scheme 3-1).



Scheme 3-1. Biooxidation of substrates containing benzylic chiral centers

To date, there are only five instances of biooxidation of such compounds have been reported. Gibson *et al.* reported the production of diol **337** in conjunction with their studies of di- vs. monohydroxylation.<sup>333</sup> The study of this substrate has been further elaborated by Cripps and co-workers.<sup>334</sup> Several studies were recently conducted by

Boyd *et al.* on 1-phenylethan-1-ol and other substrates.<sup>335</sup> Ribbons and Ahmed<sup>336</sup> studied the biooxidation of phenyl ethanol (*R*-, *S*-, and racemate) with blocked mutants and reported a slight preference for the *S*-enantiomer. Racemic *sec*-butylbenzene **338** and racemic 1-phenylpropan-1-ol also were oxidized, but the stereochemical outcome was not reported. In view of these limited examples, several substrates with one or more remote chiral centers were oxidized by means of parallel experiments with both blocked mutants *P. putida* F39/D and recombinant organism, *E. coli* JM109 (pDTG601) to investigate the kinetic resolution capability of TDO in resolving enantiomer pairs.<sup>29,200,337</sup> The following sections summarized the results obtain from this kinetic resolution study. The absolute stereochemistry correlation of new metabolites are also presented.

At the onset of this study, Ribbons and Ahmed's experiments with phenethyl alcohol were repeated as control experiments to ensure the viability of *P. putida* F39/D and to verify the kinetic resolution they observed. When phenylethanol was inoculated with toluene-induced *P. putida* F39/D, the *S*-enantiomer was observed to be consumed at a faster rate than the *R*-antipode resulting in a 6:4 ratio of diastereomers **337a** and **337b**, respectively, as confirmed by <sup>1</sup>H-NMR spectroscopy and chiral HPLC analyses of the metabolites formed (Table 3-1). This preliminary finding suggested that TDO is capable of resolving racemic enantiomers and encouraged further investigation into the scope of its kinetic resolution capacity.

Since the recombinant organism *E. coli* JM109 (pDTG601) is easier to handle and grow, further investigation of TDO's kinetic resolution capability was conducted with the *E. coli* strain. When racemic phenylethanol **339** was subjected to TDO dihydroxylation with JM109 (pDTG601), both enantiomers were converted to a 1:1 mixture of

diastereomers **337a** and **337b**, as revealed by both  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy. In experiments where the biooxidation process was terminated before completion, analyses of the non-converted phenylethanol on a chiral support GLC revealed no difference in the rate at which each enantiomer was metabolized. Moreover, in runs where the *R*- and the *S*-enantiomers were oxidized separately, the same phenomenon was observed. These results were unexpected, since the same dioxygenase enzyme operates in both *P. putida* F39/D and JM109 (pDTG601). Table 3-1 summarized the biotransformation results of phenylethanols by both organisms.<sup>330,331</sup>

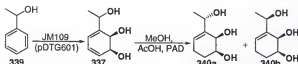
Table 3-1. Biotransformation of phenylethanol by JM109 (pDTG601) and *Pseudomonas putida* F39/D

| Substrates                                                                        | <i>E. coli</i> JM109 pDTG601)                                                      |                                                                                   | <i>Pseudomonas putida</i> F39/D                                                    |                                                                                   |
|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
|  |   |  |   |  |
| 339                                                                               | 337a                                                                               | 337b                                                                              | 337a                                                                               | 337b                                                                              |
|                                                                                   | (1                                                                                 | : 1)                                                                              | (6                                                                                 | : 4)                                                                              |
|  |   |                                                                                   |   |                                                                                   |
| 339a                                                                              | 337a                                                                               |                                                                                   | 337a                                                                               |                                                                                   |
|  |  |                                                                                   |  |                                                                                   |
| 339b                                                                              | 337b                                                                               |                                                                                   | 337b                                                                               |                                                                                   |

In light of the observed differences between the two organisms in oxidation of phenylethanol, it was speculated that each organism might have different substrate transport mechanisms. For example, in the *P. putida* F39/D, it is possible that the *S*-enantiomer was transported to the enzyme active site at a faster rate than the *R*-antipode, resulting in its consumption at a faster rate. By contrast, in the *E. coli* organism, the rate

of transport for both enantiomers maybe equal, which would result in both enantiomers being metabolized at the same rate. This result also could be rationalized by slight differences in the enzyme topology between the two organisms, perhaps resulting from post-translational folding.

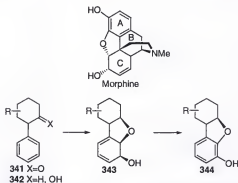
The absolute stereochemistry of metabolites **337a** and **337b** derived from biooxidation of 1-phenyl-1-ethanols has been established<sup>333,334,336</sup> and the diimide reduction of the metabolites **337a** and **337b** via potassium azodicarboxylate (PAD) to **340a** and **340b**, was carried out to confirm a diastereomeric 1:1 ratio of the metabolites (Scheme 3-2).



Scheme 3-2. Biooxidation of racemic phenylethanol by JM109 (pDTG601)

The biooxidation of phenylethanol in both racemic and enantio-pure forms provided us with a glimpse at the kinetic resolution power of TDO. During this preliminary study, no evidence of enantiodiscrimination was found, except perhaps a slight preference for the oxidation of the S-enantiomer **339a** of 1-phenyl-1-ethanol when the blocked mutant *P. putida* 39D was used. On the other hand, no preference was detected in the oxidations mediated by TDO expressed in the recombinant organism *E. coli* JM109 (pDTG601). Nonetheless, it was envisioned that if the size of the substituents at the benzylic stereogenic center of substrate were to increase, the enzyme would be more discriminatory in its kinetic resolution of racemic enantiomers. We reasoned that phenyl-cyclohexanone **341** and phenylcyclohexanol **342**, would be ideal for this task as

they would, if successfully oxidized, furnish “resolved” synthons that would be useful in construction of the dibenzofuran core of the morphine alkaloid (Scheme 3-3).

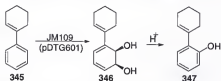


Scheme 3- 3. Chemoenzymatic approach to morphine synthesis--introduction

#### Biotransformation and Absolute Stereochemistry of New Metabolites

##### Phenylcyclohexene

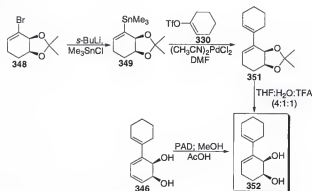
Before such undertaking was carried out, simpler substrates of similar structural motif of **341** and **342** were screened to test for TDO's tolerance of such structural feature. Phenylcyclohexene **345** was chosen for this experiment because of its having a structure similar to the morphine model substrates **341** and **342**. Biooxidation of



Scheme 3-4. Biooxidation of 1-phenylcyclohexene

phenylcyclohexene was carried out initially in a shake-flask experiment, as described in the experimental section and metabolite **346** was detected by UV ( $\lambda_{\text{max}}=302$  nm). Full-scale fermentation of phenylcyclohexene was implemented in a 15-L fermentor to

generate **346** in a crude yield of 3 g/L (98% conversion) as an off-white solid.<sup>330,331</sup> Diol **346** was observed to be acid and thermal unstable and re-aromatized readily to **347** at room temperature (Scheme 3-4). For characterization of the new metabolite, it was necessary to convert the triene diol **346** to a more stable derivative. This was achieved by subjecting triene diol **346** to diimide reduction with PAD to furnish a much more stable diol **352**. Absolute stereochemistry of **346** therefore was established indirectly through the chemical synthesis of **352**. This was accomplished through coupling vinyl bromide **348**, whose absolute stereochemistry is known,<sup>338</sup> via its trimethylstannane derivative **349** with cyclohexanone enol triflate **350**<sup>339</sup> that was derived from cyclohexanone through a palladium-catalyzed Stille coupling process<sup>340</sup> (Scheme 3-5). The resulting coupled product **351** was treated with THF:H<sub>2</sub>O:TFA (4:1:1) mixture of reagents to furnish **352** whose physical data matched with that of the compound derived from fermentation of phenylcyclohexene, and established the absolute stereochemistry of metabolite **346** as depicted in Scheme 3-5.

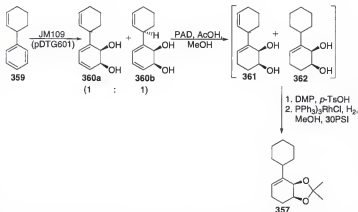


Scheme 3- 5. Absolute stereochemistry correlation of metabolite **346**



## 2-Phenylcyclohexene

2-Phenylcyclohexene was another substrate of TDO successfully oxidized during this kinetic resolution study. The biooxidation of substrate **359** furnished a 1:1 mixture of diastereomers **360a** and **360b**, which were subjected to diimide reduction to reduce the least sterically hindered olefins. However this process only provided an inseparable mixture diol **361** and **362** (Scheme 3-8). To transform this diastereomer mixture of **361** and **362** into intermediate **357**, the biochemically installed cis-diols of the mixture were protected as acetonide and Wilkinson's catalyst was used to reduce the unconjugated olefin to furnish **357** whose absolute stereochemistry has been established as described in Scheme 3-7.

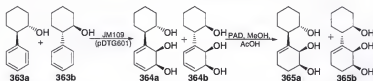


Scheme 3-8. Biooxidation of 2-phenylcyclohexene and absolute stereochemistry of new metabolite

## 2-phenylcyclohexanol

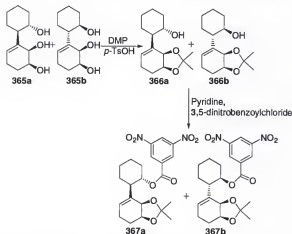
At this point it was clear that the cyclohexyl-substituted arenes are acceptable substrates for TDO and that both enantiomers were processed by the enzyme to corresponding diastereomers. *Trans*-2-phenylcyclohexanol **363**, which has two

neighboring stereogenic centers that closely resemble the intended morphine models **341** and **342**, was converted to the corresponding dienediol as a 1:1 mixture of diastereomers **364** without any observable preference of the enzyme for either enantiomers. The diastereomers **364** were reduced to **365** for characterization (Scheme 3-9).



Scheme 3-9. Biooxidation of trans-2-phenylcyclohexanol **343**

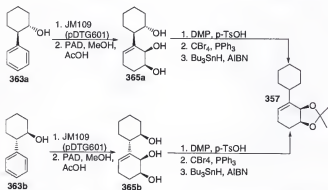
Attempts to separate **365a** and **365b** via fractional recrystallization, flash-chromatography, and derivatization of the reduced triols were unsuccessful. Derivatives **367a** and **367b** were generated via protection of the biochemically installed cis-diol as ketal followed by acylation of the free hydroxyl group. This produced a white solid



Scheme 3-10. Derivatization of triols **365** for separation

mixture of **367a** and **367b** that were inseparable by conventional chemical techniques, (Scheme 3-10).

In order to characterize these metabolites individually, biotransformation of the individual enantiomers was necessary. In two separate experiments, **363a** and **363b** were individually oxidized and metabolites were separately reduced with PAD to triol **365a** and **365b**, respectively (Scheme 3-11). The biochemically installed *cis*-diols were protected as acetonide, and the resulting free alcohols were individually treated with  $\text{CBr}_4$



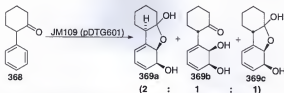
Scheme 3-11. Absolute stereochemistry of new metabolites obtained from

and  $\text{PPh}_3$  followed by  $\text{Bu}_3\text{SnH}$  to furnish **357**, whose absolute stereochemistry had been established, as described in Scheme 3-7. Thus, the chemical manipulations shown in Scheme 3-11 established the absolute stereochemistry of the metabolites obtained from biooxidation of *trans*-2-phenylcyclohexanol.

## 2-Phenylcyclohexanone

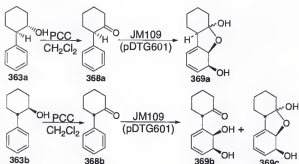
When biotransformation of racemic 2-phenylcyclohexanone was carried out, an inseparable mixture of metabolites **369a**, **369b**, and **369c** in a ratio of 2:1:1 was isolated

(Scheme 3-12). Characterization of this product mixture was complicated by the formation of the hemiketal **369a** and **369c**. In order to get a better picture of this



Scheme 3-12. Biotransformation of 2-phenylcyclohexanone

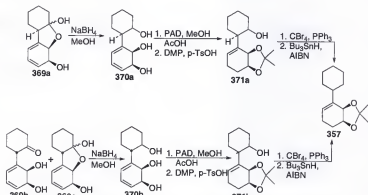
biotransformation process, pure enantiomers of **368a** and **368b**, obtained from pyridinium chlorochromate (PCC) oxidation of commercially available alcohols **363a** and **363b**, were each oxidized separately with JM109 (pDTG601) (Scheme 3-13). Ketone **368a**, upon oxidation, formed hemiketal **369**, while ketone **368b** gave a mixture of ketone **369b** and hemiketal **369c**.



Scheme 3-13. Biotransformation of enantiomerically pure 2-phenylcyclohexanone

The absolute stereochemistry of the newly formed metabolites **369a**, **369b**, and **369c** were established by first reducing the diastereomer mixtures with  $\text{NaBH}_4$  in MeOH to afford a mixture of diastereomers of alcohols **370a** and **370b**. The least hindered olefins in **370a** and **370b** was reduced with PAD and the *cis*-diol moiety was protected as

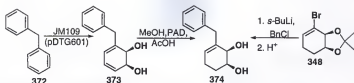
a ketal using dimethoxypropane and a catalytic amount of *p*-toluene sulfonic acid to give alcohol **371a** and **371b** (Scheme 3-14). The alcohols were treated with  $\text{CBr}_4$  and  $\text{PPh}_3$  to generate corresponding bromides, which were reduced with  $\text{Bu}_3\text{SnH}$  to give **357** whose spectroscopic and physical data were in accord with its synthetic version as disclosed in Scheme 3-7.



Scheme 3-14. Absolute stereochemistry of metabolites obtained from biotransformation

### Diphenylmethane

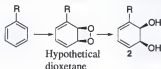
The biotransformation of diphenylmethane **372** was carried out according to the general procedure for large scale biotransformation in an 8 L fermentor to furnish 1.1 g/L (89% conversion) of metabolite **373** as an off-white solid. Absolute stereochemistry of **373** was established by first reducing the electron rich olefin with PAD to diol **374**, which was chemically synthesized by coupling vinyl bromide **348** with benzylchloride using *s*-BuLi as the metal halogen exchange reagent, followed by deprotection of the acetonide under acidic condition to arrive at compound **374** (Scheme 3-15).



Scheme 3-15. Biooxidation of diphenylmethane

### Biooxidation of Cyclopropylbenzene

The remarkable ability of TDO to process various aromatic substrates has for years been a subject of study involving scientists from many disciplines. Today much progress has been made in gaining better understanding of the enzyme's properties, including substrate specificity, regioselectivity, and kinetic resolution capacity. However, many mechanistic aspects of the enzyme functions remain obscured and unresolved.

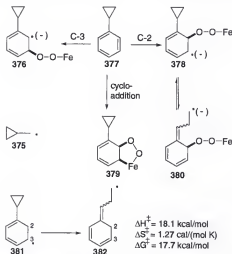


Scheme 3-16. Proposed reaction sequence for the formation of cis-dienediol

Early studies by Gibson and colleagues suggested that a hypothetical dioxetane was an intermediate, which would account for the *cis*-configuration of diol **2** found in metabolites from biotransformation by prokaryotic organisms (Scheme 3-16). This proposal was supported by  $^{18}\text{O}$  incorporation experiments where both atoms of oxygen in the metabolite were shown to come from molecular oxygen.<sup>202</sup> The pathway proposed for the oxidation of benzene by *P. putida* is given in (Scheme 2-21). However, the minute details surrounding the oxygen incorporation step, *i.e.* whether or not the process goes through a radical intermediate, has not been deciphered.

### Mechanistic Investigation

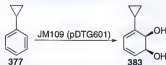
To probe for a better understanding of this mechanistic aspect of TDO, cyclopropylbenzene was chosen as model substrate for tracking radical intermediates if such species formed during the biotransformation process. If the biotransformation process goes through a radical intermediate of type **378**, there is a chance that it could



Scheme 3-17. Mechanistic possibilities for cis-dihydroxylation

lead to the fragmentation of the cyclopropyl ring to form the radical anion **380** (Scheme 3-17). Such fragmentation would be likely to occur, since cyclopropylcarbinyl type rearrangements have been observed during the investigation of the mechanism involved in the isopenicillin N synthase reaction<sup>343-346</sup> and Iron IV-sulfur-bound species<sup>347</sup> have been proposed to account for the results where trapping of the butenyl radical by the sulfur atom have been observed.

When cyclopropylbenzene was subjected to dihydroxylation with JM109 (pDTG601), only *cis*-dienediol **383** metabolite was isolated and no trace of the cyclopropyl-ring opened product was observed (Scheme 3-18).



Scheme 3- 18. Biotransformation of cyclopropylbenzene

This intriguing result prompted us to investigate and compare the reactive tendency of a conjugated cyclopropylcarbiny radical such as **378** to the well-known cyclopropylcarbiny radical **375** rearrangement<sup>348-350</sup> through computational analysis using the unrestricted Becke's hybrid three-parameter functional (UB3LYP).<sup>351</sup> This method provides a reliable level of theory for examining the cyclopropylcarbiny radical system,<sup>352</sup> and the density functional theory methodology was used to examine the kinetics of the ring opening of the cyclopropylcarbiny radical system **381** to the conjugated butenyl system **382** (Scheme 3-17). Calculations were performed using the Gaussian 98 program package.<sup>353</sup> Structures were optimized and vibrational frequency calculations were performed using UB3LYP level of theory and the 6-31G(d) basis set.<sup>354</sup> The transition structure was characterized by a single imaginary frequency, and thermochemical information was obtained using unscaled frequencies. Single-point energies were calculated using UB3LYP level of theory using the 6-311+G(2df,2p) basis set.<sup>355</sup>

At 35 °C the ring opening of the cyclopropylcarbiny radical, **375**, ( $k_r = 1.4 \times 10^8 \text{ s}^{-1}$ )<sup>356-359</sup> is  $10^6$  times faster than that of radical **381** ( $k_r^{\text{calc}} = 1.1 \text{ s}^{-1}$ ). Radical stabilizing

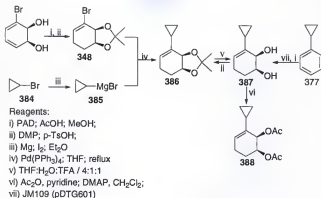
substituents at the carbinyl position are known to retard the rates of ring opening.<sup>360</sup> Even if a radical is formed at C3, ring opening may not occur if the radical has a short lifetime.

From the computational calculations it appeared that a radical of type **381** would be very unlikely to participate in a ring opening reaction and this is supported by experimental results as evidenced by the fact that no open-ring product formed during the biotransformation process.

The calculation described here for species, such as **378**, however, does not eliminate the possibility of intermediates of this type in the oxidation of cyclopropyl benzene even though products derived from the ring opening of the cyclopropane have not been observed in the reaction mixtures. While the nature of the *cis*-dihydroxylation remains a mechanistic enigma, the recent evidence by Gibson points to a possible stepwise mechanism for this unique reaction. Whether the mechanism involves radical or anionic iron-bound species is at the moment unknown.

### Absolute Stereochemistry Correlation of Cyclopropyldiol **383**

The absolute stereochemistry of diol **383** was confirmed by an independent synthesis of vinylcyclopropane **387** from bromodienediol whose absolute stereochemistry is known (Scheme 3-19). Compound **387** was prepared by the selective reduction of diol **383** with potassium azadicarboxylate (PAD) in 73% yield. Bromodienediol also was reduced with PAD (86%) and protected to provide acetonide **348** (78%).<sup>361</sup> The Grignard reagent, **385**, derived from cyclopropyl bromide **384** was coupled with **348** via a modified Corey-House synthesis<sup>362</sup> to give vinylcyclopropane **386** in a low yield (16%). Deprotection of this material with THF:H<sub>2</sub>O:TFA/4:1:1 afforded diol **387** whose  $\alpha_D$  (-126°) matched that of the material obtained via fermentation of cyclopropylbenzene.



Scheme 3-19. Absolute stereochemistry correlation of metabolite obtain from biotransformation of cyclopropylbenzene

### Synthetic Application Potential of Cyclopropylbenzene Metabolite

From a synthetic viewpoint the cyclopropyl system offers a wide variety of opportunities for further synthetic manipulations and can be viewed as a chiral vinylcyclopropane synthon for incorporation into future synthetic endeavors (Figure 3-1).

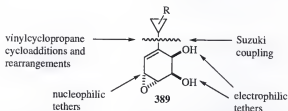
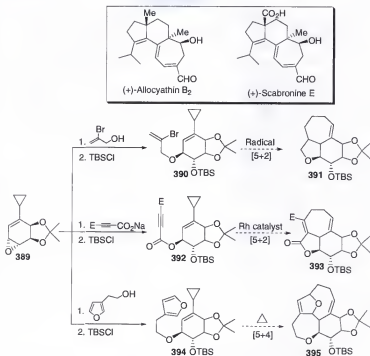


Figure 3-1. Exploration of reactivity of oxygenated cyclopropylhexanyl systems

Two obvious applications are the tethered [5+2] annulation to 7-membered ring systems, discovered and developed by Wender,<sup>363-369</sup> and the simple thermolysis to cyclopentenes,<sup>370</sup> or the cascade radical cyclizations with concomitant unraveling of the

cyclopropylcarbinyl system. These cascade reactions could offer a nice approach to tricyclic ring system for construction of allocyathin B<sub>2</sub> and scabronine E as depicted in Scheme 3-20.



Scheme 3- 20. Synthetic potential of cyclopropyl epoxide **389**

### Substituted Catechols

The catechol unit, free or alkylated, is a ubiquitous component of countless natural products. Morphine,<sup>261,371</sup> narciclasine,<sup>372</sup> pancratistatin,<sup>245,246,373</sup> combretastatins A-1<sup>291,374</sup> and B-1<sup>291</sup> are a few of such natural products that are of great interest within the Hudlicky's research program because of their extraordinary architectures and interesting

biological activities. Morphine, for instance, is structurally small but its architecture complexity has been an intriguing challenge for many synthetic chemists.

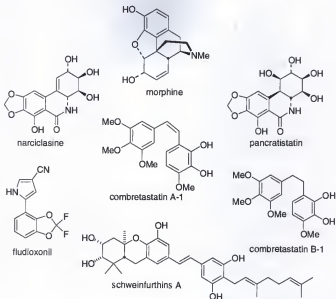


Figure 3-2. Oxygenated natural products containing a catechol subunit

Syntheses of simple catechols have been reviewed.<sup>375</sup> For the most part, the available chemical methods involve the cleavage of mono- or dialkylated catechols by strong protic or Lewis acids under relatively harsh conditions.<sup>376-378</sup> Other methods use strong oxidants<sup>379-381</sup> for direct hydroxylation of aromatic nucleus or cleavage of mono- and diacetates.<sup>382,383</sup> The arduous, non-selective and low-yielding difficulties associated with these methods have hindered their widespread usage. Despite the effort invested, there exists no reliable method for direct generation of catechols through conventional

chemical methods. As a result, this class of compounds continue to elude synthetic chemists and their non-trivial preparation is reflected in the pricing (Figure 3-3).

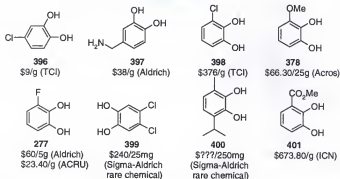


Figure 3-3. Some commercially available catechols

The mild enzymatic approaches to catechols, on the other hand, are limited and under-explored with only three such methods having been reported in the literature.<sup>29,384,283</sup> Among these, Frost's enzymatic synthesis of the parent catechol from glucose via an engineered aromatic amino acid pathway is one of the most noteworthy. This example demonstrates the power of biocatalysis in the synthesis of commodity chemicals.

With the above limited information, we set out to explore the practical implication of whole cell fermentation with *E. coli* JM109 (pDTG602) for generation of substituted catechols as an environmentally benign and straightforward preparative procedure. This organism, which was developed by D.T. Gibson in 1989, allows for an approach to the whole-cell biotransformation of aromatic substrates to corresponding catechols. The genetic make up that is responsible for such transformation came from the *Pseudomonas* species whose metabolic pathway of aromatic degradation was elucidated by Gibson and

co-workers in the late 1960's (Scheme 1-1). The genes responsible for the production of TDDH in *P. putida* F39D were cloned and expressed in the *E. coli* JM109 (pDTG602) host. Although the organism has been in existent for more than a decade, its use has been

Table 3-2. Catechols From Fermentation of Corresponding Arenes with JM109 (pDTG602)

| <br>277<br>[2g/L]    | <br>398<br>[1.5 g/L] | <br>270<br>[1.2 g/L] | <br>402<br>[0.5 g/L] | <br>98<br>[2.2 g/L] |
|----------------------|----------------------|----------------------|----------------------|---------------------|
| <br>279<br>[0.5 g/L] | <br>403<br>[1.0 g/L] | <br>404<br>[1.2g/L]  | <br>405<br>[1.2g/L]  | <br>406<br>[0.5g/L] |
| <br>407              | <br>400<br>[0.4 g/L] | <br>408<br>[0.07g/L] | <br>409<br>[0.35g/L] |                     |

Notes: Numbers in brackets denote the not optimized isolated yield of metabolite in g/L.

almost non-existent, despite its great potential as a synthetic tool. This is surprising in view of the importance of simple catechols as synthetic building blocks, the common occurrence of these functionalities in natural products, and the difficulty associated with their synthesis in polyfunctional molecules.

Recognizing the importance of substituted catechol in synthesis, we set out to investigate the capability of *E. coli* JM109 (pDTG602) to generate substituted catechols. The biotransformation of various substituted arenes with *E. coli* JM109 (pDTG602) is summarized in Table 3-2. The results illustrate the broad substrates specificity of the enzyme as well as its excellent regioselectivity.

The size of the substituents on the aromatic ring seems to play a role in the metabolism of the substrate by TDDH. The smaller the substituents the better the substrate it is for the enzyme, thus affording higher yields. The bulkier the substituents on the ring, the lower the yield of the metabolites. Although the yields reported in Table 3-2 appear to be low, they are a lot better when compared with the yield from multi-step syntheses of similar catechols through conventional chemical methods. Comparison of efficiency of non-enzymatic and biocatalytic syntheses is shown in Table 3-3. The overall chemical yields are indicated as reported in the literature. The E value<sup>385</sup> is calculated by dividing the weight of all materials used in synthesis by the weight of product. The EMY<sup>386</sup> is calculated by expressing the weight of product as a percentage of all non-benign mass used in the manufacturing. Solvents used for extractions are not taken into account in either calculation because they would be recycled during manufacturing. It is obvious that the EMY values are more informative about the efficiency of the process than the overall yields, because the latter do not provide any information about the process itself. It is also evident that the single step biooxidation is far more efficient than traditional chemical preparation, even though the yield is limited by the concentration of product at 1-2 g/L, beyond which it is toxic to the organism.<sup>283</sup>

Table 3-3. Comparison of efficiency of the enzymatic vs. non-enzymatic preparation of 3-bromo-, 3-methyl-, 3-iodocatechols

| Catechols                                                                                | Chemical                                                                                                                                                    | Enzymatic                                              |
|------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|
| <br>270 | Overall yield = 35 % <sup>a</sup> ; 52 % <sup>b</sup><br>E value = 47.50 <sup>a</sup> ; 36.25 <sup>b</sup><br>EMY = 2.1 % <sup>a</sup> ; 2.8 % <sup>b</sup> | Overall yield = 63 %<br>E value = 1.25<br>EMY = 75.0 % |
| <br>98  | Overall yield = 1 % <sup>c</sup><br>E value = 63.50 <sup>c</sup><br>EMY = 1.6 % <sup>c</sup>                                                                | Overall yield = 45 %<br>E value = 2.25<br>EMY = 60.0 % |
| <br>402 | No synthesis                                                                                                                                                | Overall yield = 34 %<br>E value = 2.0<br>EMY = 38 %    |

Note: Superscripts denote previous synthesis of corresponding catechols. <sup>a</sup> Ref.<sup>387</sup>

<sup>b</sup> From procedure in Ref.<sup>388</sup>, <sup>c</sup> From procedure in Ref.<sup>274</sup>

Since TDO is the enzyme that precedes catechols dehydrogenase in the aromatic degradation pathway of *P. putida*, the regioselectivity of TDO presets the regiochemistry of catechols produced in the process. As with TDO, the hydroxylation sites for JM109

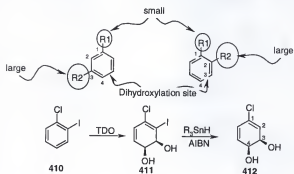
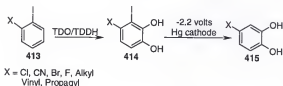


Figure 3-4. Chemoenzymatic approach to 3,4-cis-dihydrodiols via directing

(pDTG602) have been found exclusively at the 1,2-positions for mono-substituted aromatic substrates. For 1,2- or 1,3 disubstituted substrates, the hydroxylation sites can be predicted using Boyd's model in his approach 3,4-*cis*-diols (Figure 3-4).

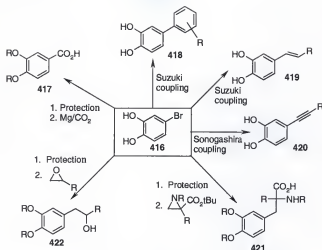
Based on the predictions of the above-mentioned model, in which the bulkiest substituent of the ring directs the hydroxylation sites, substrates can be designed to generate 2,3- or 3,4-catechols. The versatility of this method could allow investigators to gain access to a variety of important catechols that otherwise would be impossible to obtain through chemical means. Such catechols would facilitate synthesis of a variety of naturally occurring amino acids, aromatic antibiotics, and many isoquinoline and quinoline alkaloids.

For instance, substrates such as **413** (Scheme 3-21) can be designed to provide 3,4-functionalized catechols following the electrochemical reduction of the directing halogen. Such regiochemistry is commonly found in naturally occurring amino acids, aromatic antibiotics, and many isoquinoline and quinoline alkaloids.



Scheme 3-21. An environmentally benign approach to 3,4-catechols through

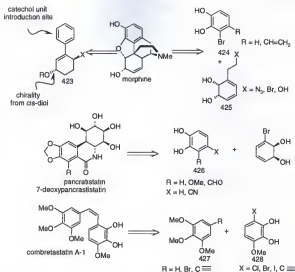
The 3,4-catechol class of compounds, such as 1-bromo-3,4-catechol **400** as shown in Scheme 68, created through this technique can be used in a variety of chemical transformations.



Scheme 3-22. Transformations of 3,4-bromocatechol

The availability of catechols and *cis*-diols produced through enzymatic means would allow for an easy assembly of synthetic targets such as morphine, pancratistatin, and combretastatins as shown in Scheme 3-23.

As demonstrated, functionalized catechols can be prepared directly from their mono- or disubstituted aromatic precursors by biooxidation on a preparative scale. Such compounds represent important starting materials for synthetic routes to many natural products and are sometime unattainable by traditional methods (for instance iodocatechol). The halogenated catechols are useful for the introduction of catechol moiety into those synthons that rely on Heck cyclization or tethered radical for C-C bond formation. Future direction of this research will clearly be concerned with the screening of a broad range of substrates for the recombinant organism and establishment of the substitution limits for recognition by the enzymes.



Scheme 3- 23. Retrosynthetic design of catechol (or arene-*cis*-diol) synthons

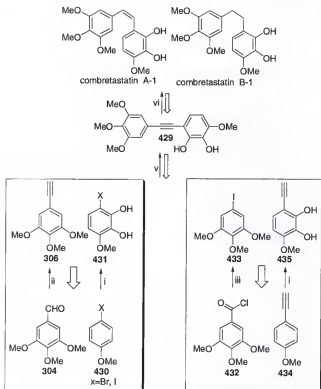
### Chemoenzymatic approach to combretastatins A-1, and B-1

Oxygenated natural products containing a catechol subunit are ubiquitous in nature. Combretastatins isolated from the bark of an African willow tree *Combretum caffrum*<sup>291</sup> have such moiety embedded in their structures (Figure 2-12). Biological studies of these compounds have revealed that they are among the most cytotoxic agents tested so far against a series of cancer cell lines.<sup>302,313,389,390</sup> They share a common binding site on tubulin with the well-known antimetabolic agents colchicine, podophyllotoxin, and stegananin and are capable of inhibiting microtubule assembly at relatively low concentrations (Table 2-16).<sup>291</sup> The fact that combretastatin A-1 is capable of displacing 97.8% of colchicine from its binding site at 2  $\mu$ M concentration shows that it is by far the best antimetabolic agent compared with previously known drugs. The high potency of

combretastatins as angiogenesis inhibitors offers new hope and approach to cancer treatment.<sup>391</sup>

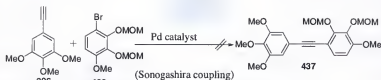
Because of their valuable biological activity, much effort has been invested in the synthesis of these natural products, especially combretastatin A-1 and A-4, and their derivatives. Early approaches to the combretastatins utilize the Wittig coupling reaction of **294** and **295** as the key step to install the olefin flanking the two aromatic portions of the natural products.<sup>291</sup> This approach gives rise to a mixture of *cis*- and *trans*-isomers of combretastatin A-1 which are difficult to separate (Scheme 2-37).

In our approach to this problem, it was envisioned that the catechol moiety of the natural products could be constructed by the TDDH carrying organism, *E. coli* JM109 (pDTG602).<sup>200</sup> Oxidation of the para-substituted anisoles such as **430**, and **434** using *E. coli* JM109 (pDTG602) would, in one operation, generate the functionalized catechol unit of combretastatin A-1 and B-1. We envisioned that palladium catalyzed Sonogashira or Suzuki coupling of the catechol moiety with the appropriate 3,4,5-trimethoxy unit as illustrated in Scheme 3-24 would provide the natural products main skeleton as an internal alkyne **429**. Selective hydrogenation of **429** via Lindlar's catalyst would provide *cis*-alkene, combretastatin A-1, or by Pd/C/H<sub>2</sub> would provide the fully saturated alkane, combretastatin B-1 (Scheme 2-24). Of the potential substrates for this task, **430**, and **434**, only 4-bromoanisole was successfully oxidized to the corresponding catechol with a yield of 1.2g of catechol per L of fermentation broth. Bromocatechol was then used to assemble combretastatin A-1 and B-1.



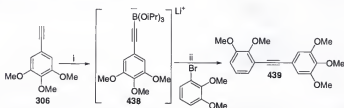
Scheme 3-24. Chemoenzymatic retrosynthetic possibilities to combretastatins

Initially, Sonogashira coupling conditions<sup>392-396</sup> were used in an attempt to couple trimethoxyacetylene **306** and **436**. This approach failed to give any desired coupled product **437** (Scheme 3-24), and starting materials were recovered unchanged.



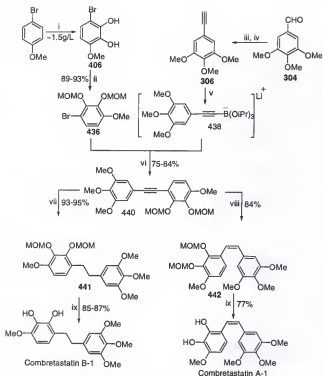
Scheme 3-25. Sonogashira coupling approach to combretastatins

A variation of Suzuki coupling reaction between 3,4,5-trimethoxyacetylene **306** and dimethoxybromocatechol **438** was attempted as a model study (Scheme 3-26). The coupling reaction proceeded successfully to afford **439**, which offers a direct avenue for construction of combretastatin A-1 and B-1.<sup>397-401</sup>



Conditions: i)  $n\text{BuLi}$ ,  $\text{B}(\text{OiPr})_3$ , DME:THF/10:1,  $-78^\circ\text{C}$ ; ii)  $\text{Pd}(\text{PPh}_3)_4$ , DME:THF/10:1, Reflux  
Scheme 3-26. Suzuki approach to combretastatins

In our approach to combretastatin A-1 and B-1, *p*-methoxybromocatechol **406** prepared through bio-oxidation of the corresponding arene, was first functionalized as MOM-protected derivative **436** and then subjected to Suzuki-Miyaura coupling conditions with alkynylboronic ester of **438** to form internal alkyne **440**. Hydrogenation of **440** using 10% Pd/C at 30 PSI for 30 minutes afforded the MOM-protected combretastatin **441** in 93-95% yield. Acid hydrolysis of **441** in a 1:1 mixture of 3N HCl:THF solution provided combretastatin B-1, in an overall yield of 52% over 4 steps, excluding the biotransformation process. The spectroscopic data for combretastatin B-1 were in accord with the literature values<sup>402</sup> (Scheme 3-27).



**Conditions:** i) JM109 (pDTG602); ii) MOMCl;  $\text{CH}_2\text{Cl}_2$ ;  $\text{EtN}(\text{IPr})_2$ ; iii)  $\text{OsO}_4$ ;  $\text{PPh}_3$ ;  $\text{CH}_2\text{Cl}_2$ ;  $0^\circ\text{C}$ ; iv)  $n\text{BuLi}$ ; THF; v)  $n\text{BuLi}$ ,  $\text{B}(\text{O}i\text{Pr})_3$ , DME:THF/10:1;  $-78^\circ\text{C}$ ; vi)  $\text{Pd}(\text{PPh}_3)_4$ ;  $90^\circ\text{C}$ ; vii) 10%  $\text{Pd}/\text{C}/\text{H}_2$ ; (30 PSI); MeOH; viii)  $(\text{C}_6\text{H}_{11})\text{BH}$ ,  $0^\circ\text{C}$ , 1h; then  $20^\circ\text{C}$ , 30 min; HOAc;  $0^\circ\text{C}$ , 4h; ix) 6N HCl; THF/1:1

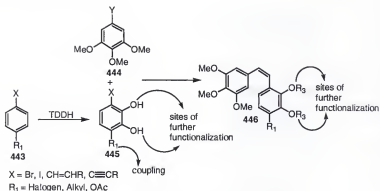
Scheme 3-27. Enzymatic generation of catechol for approach to combretastatin A-1 and B-1

In the preparation of combretastatin A-1, alkyne **440** was initially subjected to Linlard's reduction condition to selectively reduce the alkyne to alkene. However, this method was not successful in generating *cis*-alkene as the sole product but rather a complex mixture of *cis*-alkene **442** and its saturated analog **441** were produced that were

inseparable by standard chromatography techniques. Therefore an alternative approach for the selective reduction of alkyne **440** to *cis*-alkene **442** was explored. It was found that hydroboration was the method of choice for such transformation to give the required *cis*-olefin **442** in 84% yield. Final acidic deprotection of the MOM-protective groups provided for a concise preparation of Combretastatin A-1 in an overall yield of 43-51% over 5 steps in a convergent manner starting from bromoanisole, (Scheme 3-27). This method is as good or better than previous literature syntheses.<sup>291,403,404</sup>

The versatility of bio-oxidation with TDO/TDDH, as demonstrated above has allowed for the generation of functionalized catechols that can be incorporated into the synthesis of natural products. Such methods also can be used to generate combretastatin derivatives as in Scheme 3-28. Catechols of type **445** may contain either carbon substituents or halogens *para* to the acetylene unit, allowing for analog synthesis through Suzuki type coupling once the combretastatin nucleus is assembled. Because iodo-, bromo-, and chloroarenes react at different rates under a variety of C-C bond forming conditions,<sup>397-401,392-396</sup> it is possible to assemble a complete combretastatin nucleus which still retains a halogen atom *para* to the styrene unit. In such a case, the R<sub>1</sub> substituent could be vary at a late stage of synthesis. On the other hand, such variations in R<sub>1</sub> may be introduced early at the stage of **443** and prior to the enzymatic oxidation. One can envision either *p*-bromoiodobenzene or *p*-bromochlorobenzene as convenient starting points for the synthesis of combretastatin analogs. The hydroxyl groups in **445** or **446** may be differentiated based on the nature of R<sub>1</sub>, and functionalized either before or after the assembly of the combretastatin nucleus. The synthesis of combretastatin analogs for optimization of their activity, as envisioned in Scheme 3-28, would be an appropriate

synthetic exercise as a follow up project once the synthesis of the parent compounds has been completed successfully.



Scheme 3-28. Chemoenzymatic approach to combretastatin analogs

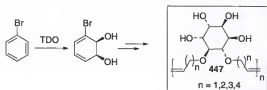
### Polyhydroxylated Chiral Polymer

Polymer chemistry is a fascinating and growing field. This branch of chemistry has attracted a great deal of attention within the last few decades as polymer usefulness becoming more and more widespread. Polymers such as plastics, fibers, elastomers, coatings adhesives, rubber, protein, and cellulose have become essential commodities within modern societies. Thanks to the seemingly endless possibilities, research activities within this field remain relentless, as the search for better materials continues.

Radical, anionic, or cationic methods for the synthesis of polymeric materials usually do not impart stereo- or enantio-selectivity to the process.<sup>405-407</sup> Chiral polymers have been of considerable interest for many years.<sup>408-410</sup> Methods for the preparation of chiral polymers remain a challenging field with many difficulties. The introduction of metal catalysts by Ziegler and Natta in the late 1940's<sup>411,412</sup> offered new approaches and

insight into how chiral polymers can be made on an industrial scale. These polymers have been found helpful as catalysts for asymmetric induction in organic synthesis,<sup>408,409,413</sup> chiral chromatographic separations,<sup>414</sup> and ferroelectric and nonlinear optical applications.<sup>415</sup> The types of synthetic chiral polymers that have been reported include the following: (1) polymers that adopt helical conformations, such as poly-(chloral), poly(isocyanates), poly(isocyanides), and poly-(triaryl methyl methacrylates); these polymers are obtained via the so-called "helix-sense-selective" polymerization from achiral monomers using either a chiral initiator or catalyst;<sup>410</sup> (2) polymers whose optical activity is derived either from main chain or side chain chirality; they are obtained by cyclopolymerization of diolefins,<sup>416</sup> diisocyanides,<sup>417</sup> divinyl ethers,<sup>418-421</sup> bis(styrenes),<sup>408,409,420,421</sup> dimethacrylates,<sup>422-425</sup> diepoxides,<sup>426</sup> and peptides<sup>427,428</sup> that mimic natural chiral polymers.

We became interested in polyhydroxylated polymers or high oligomers of type 447, (Scheme 3-29) which would be derived from the monomers of established configuration via acyclic diene metathesis (ADMET) of vicinally bis-alkylated olefinic side chains. The corresponding monomers can be obtained easily from bromodienediol, the product of the whole cell fermentation of bromobenzene with recombinant organism expressing

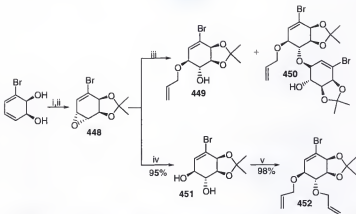


Scheme 3-29. Chemoenzymatic approach to polyhydroxylated chiral polymer

toluene dioxygenase (TDO) [*E. coli* JM109 pDTG601A],<sup>200</sup> Scheme 3-29. The configuration of the central inositol unit can be readily synthesized to reflect all nine inositols, as we have demonstrated previously.<sup>429-432</sup> The possibility also exists to approach the monomer synthesis from protected synthons – in such cases the number of isomeric possibilities in intermediates of this type would approach the theoretical limit of 64. As delineated in recent papers on combinatorial possibilities of oligoinositols,<sup>433,434</sup> the number of possible isomers increases exponentially with higher oligomers once secondary and tertiary structures are developed through hydrogen bonded forms and  $\beta$ -turns initiated by a 1,2-trans-connection in **447**.<sup>430-432,435</sup>

We were interested primarily in generating relating low molecular weight polymers and integrating physical and chemical properties of such compounds with an eye toward for the cross-linking as well as chiral separation materials.

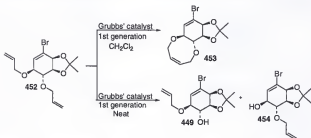
To test our approach to this problem, bis-ally ether vinylbromide **452**, (Scheme 3-30) was generated and subjected to polymerization with Grubbs' First-generation catalyst. The bis-ether was generated by first protecting the cis-diols of the bromodienediol as acetonide, followed by epoxidation of the unhindered double bond to afford epoxide **448**. The  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  catalyzed opening of epoxide **448** with allyl alcohol led to a low yield of ether **449** at the expense of generating the conduritol dimer **450**. This problem was circumvented by treating epoxide **448** first with Amberlyst resin to afford *trans*-diol **451**. The diol was then allylated with allyl bromide to afford allyl ether **452** in 98% yield (Scheme 3-30).



Conditions: i) DMP, *p*-TsOH; ii) *m*CPBA,  $\text{CH}_2\text{Cl}_2$ ; iii)  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ , Allyl alcohol;  
iv) Amberlyst Resin v) DMF, NaH, Allylbromide

Scheme 3-30. Synthesis of monomer bis-allyl ether **438**

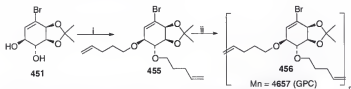
Allylether **452** was subjected to ADMET conditions with Grubbs' First-generation catalyst. When the reaction was performed in dilute methylene chloride, no polymerization occurred and only eight-member ring ether **453** formed (Scheme 3-31). When the reaction conducted as neat, again, no polymer formed but only a mixture of deallylated products **449** and **454** plus the starting material were isolated.



Scheme 3-31. Polymerization of **452** with Grubbs' First-generation catalyst

Diol **451** was alkylated with 5-bromopentene to furnish bis-allylated ether **455**.

The ether was subjected to polymerization reaction to furnish **456**, with an estimated molecular weight of 4657 (Scheme 3-32). Monomer **455** also has been polymerized using Grubbs' second-generation catalyst.

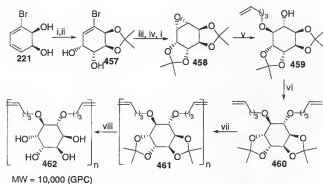


Conditions: i) DMF, NaH, 5-Bromopentene; ii) Grubbs' catalyst 1st generation

Scheme 3-32. Generation and polymerization of pentenylether **451**

The synthesis of the fully hydroxylated polymer was carried out as outline in

Scheme 3-33.



Conditions: i) DMP, *p*-TsOH; ii) Aceton, H<sub>2</sub>O, NMO, OsO<sub>4</sub>; iii) Bu<sub>3</sub>SnH, AIBN, Benzene; iv) mCPBA, CH<sub>2</sub>Cl<sub>2</sub>; v) BF<sub>3</sub>·Et<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub>, 4-penten-1-ol; vi) DMF, NaH, 5-bromo-pentene; vii) Grubbs' catalyst 1st Generation; viii) H<sup>+</sup>

Scheme 3-33. Chemoenzymatic approach to fully hydroxylated chiral polymer

In our approach to polyhydroxylated polymer **462**, the *cis*-diol **221** was first

protected and the more electron rich double bond was converted to *cis*-diol **457**.

Debromination of **457** was achieved with tributyltinhydride and subsequent epoxidation

with *m*-CPBA followed by protection of the cis-diol provided epoxide **458**.  $\text{BF}_3\text{-Et}_2\text{O}$  catalyzed epoxide opening with 4-penten-1-ol gave alcohol **459**, which was alkylated with 5-bromopentene to furnish the desired monomer **460**. Exposure of this monomer to Grubbs' catalyst (neat) gave polymer **461**, which upon deprotection under acidic condition gave rise to the fully hydroxylated polymer **462**, with MW estimated at ~10,000 via GPC.

## CHAPTER 4

### CONCLUSIONS

Toluene dioxygenase (TDO) is a powerful and excellent enzyme system that has great potential for synthetic application. Using a combination of enzymatic oxidation and chemical manipulation of the metabolites from TDO have allowed us to construct a variety of highly oxygenated natural products and their derivatives. A series of bicyclic substrates with and without remote chiral centers was oxidized and the enzyme was found to be nondiscriminatory toward enantiomers. Cyclopropylbenzene was oxidized to probe for mechanistic details of the biotransformation process. The *E. coli* JM109 (pDTG602) was used successfully for the generation of substituted catechols that can be used in synthetic applications. For example, combretastatin A1 and B1 were successfully synthesized

## CHAPTER 5 EXPERIMENTAL SECTION

### General Methods

All non-hydrolytic reactions were carried out in an argon atmosphere with standard techniques for the exclusion of air and moisture. Glassware used for moisture sensitive reactions was flame dried under reduced pressure or dried overnight at 80 °C. Tetrahydrofuran, benzene, and diethyl ether were distilled from sodium benzophenone ketyl. Dichloromethane was distilled from calcium hydride. Microbial oxidations were carried out in a 15 L B. Braun Biostat E fermentor and B. Braun Biostat D. All  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded at 300 and 75 MHz, respectively, using a Varian 300 spectrometer, unless otherwise stated. Chemical shifts are reported relative to TMS,  $\text{CDCl}_3$  or  $\text{DMSO}-d_6$ . Coupling constants are measured in Hz. Multiplicities in  $^{13}\text{C}$  NMR are based on APT or DEPT. IR spectra were recorded on a Perkin–Elmer FT-IR (KBr) and are given as wavenumbers in  $\text{cm}^{-1}$ . Mass spectra are given with relative abundance in parentheses. High resolution mass spectroscopy (HRMS) and elemental analyses were performed at the University of Florida and Atlantic Microlab, Inc, respectively. Optical rotations were recorded on the Perkin–Elmer 241 digital polarimeter ( $10^{-1}$  deg.  $\text{cm}^2 \text{g}^{-1}$ ). Melting points were obtained on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Analysis of enantiomeric mixtures was performed on a cyclodextrin column (Chirasil DexCB, 25m x 0.32 mm with helium as the carrier gas). Reversed phase HPLC analyses were performed on a C18 column (Phenomenex 250x10 mm) using

mixtures of water and acetonitrile as eluents. For chromatographic purification, silica gel (grade 60, 230–400 mesh Merck) was used.

#### Large Scale Biotransformation - *E. coli* JM109 (pDTG601)

A 600-mL of mineral salts broth (MSB) containing 9.6 g  $K_2HPO_4$ , 8.4 g  $KH_2PO_4$ , 3 g  $(NH_4)_2SO_4$ , 9 g yeast extract, 18 g glucose, 1.2 g  $MgSO_4 \cdot 7H_2O$  was prepared and divided into two 2.8 L fernbachs for sterilization. Upon cooling to ambient temperature, ampicillin (100 mg/L) was added, and the media were inoculated with single colonies of *E. coli* JM109 (pDTG 601) from an agar plate. The cells were grown overnight on an orbital shaker (35 °C, 150 rpm). The cultures were then transferred into a 15-L fermentor containing 8 L of a similar sterilized medium: 60 g  $KH_2PO_4$ , 16 g citric acid, 40 g  $MgSO_4 \cdot 7H_2O$ , 16 mL trace metal solution ( $Na_2SO_4$  (1g/L),  $MnSO_4$  (2g/L)  $ZnCl_2$  (2g/L),  $CoCl_2 \cdot 6H_2O$  (2g/L),  $CuSO_4 \cdot 5H_2O$  (0.3g/L),  $FeSO_4 \cdot 7H_2O$  (10g/L), pH =1.0), 9.6 mL concentrated  $H_2SO_4$ , 9.6 mL ferric ammonium citrate (270 g/L), neutralized with concentrated ammonia and supplemented with 800 mg of ampicillin and 2.69 g thiamine hydrochloride). The cells were grown for 24 h with concentrated glucose solution (720 g/L) as the carbon source and induced with 80 mg isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG) when the optical density (OD) of the medium is 15 or higher. When cells were grown to an OD of 45 or higher, the cultures were used for screening of new substrates or to carry out large scale biotransformation of aromatic substrates. The substrates were added to the culture and their metabolic transformation was monitored UV ( $\lambda_{max}$  ~260–276 nm) or by TLC. After all metabolic activity ceases, the fermentation was terminated and the pH of the culture was adjusted to 7.5 with ammonium hydroxide. Cells were separated from the broth by centrifugation and supernatant was extracted with base-washed ethyl acetate. The organic layer was dried with anhydrous magnesium sulfate

and the solvent evaporated. The crude diols were purified by recrystallization (hexanes:ethyl acetate) to yield the pure diols. Only minimal data ( $^1\text{H}$  NMR and/or optical rotation) were obtained for metabolites that are unstable at room temperature.

#### Screening for new substrates

One liter of induced cells ( $\text{OD}_{600} > 0.45$ ) from the fermentor was centrifuged (3000 rpm, 10 min) and re-suspended in 300 mL of 0.20 M  $\text{KPO}_4$  buffer (pH 7.0) supplemented with 2% glucose. The substrate was added in small portions while the pH was maintained at 7.0 with 10 M NaOH. The progress of the biotransformation was monitored by UV absorbance of the broth in the region of 260–270 nm and by TLC. Successfully metabolized substrates were subjected to large scale biotransformation in a 15-L fermentor.

#### Preparation of catechols

Substituted catechols were made using the same biotransformation procedures described for *E. coli* JM109 (pDTG601) except *E. coli* JM 109 (pDTG602) was used instead. All catechols were isolated by extraction of the cells-freed fermentation broth with ethyl acetate, followed by drying ( $\text{Na}_2\text{SO}_4$ ) and concentrating of the organic layers *in vacuo*. Analytical samples of catechols were obtained by distillation, recrystallization, and/or flash chromatography.

#### General procedure for diimide – potassium azodicarboxylate (PAD) – reduction of the diene diols

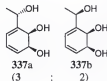
Potassium azodicarboxylate (PAD) (2.0 eq.) was added in small portions with vigorous stirring to an ice-cold solution of the diene diol (1.0 eq.) in methanol (10 mL/mmol), followed by dropwise addition of a solution of acetic acid (7.0 eq.) in methanol. The solution was allowed to slowly warm to room temperature. Saturated  $\text{NaHCO}_3$

solution was added until the solution become slightly basic (pH=8). The methanol was removed under reduced pressure and the remaining aqueous solution was extracted with ethyl acetate (4X, 30 mL). The combined organic layers were dried (MgSO<sub>4</sub>) and the solution was filtered through a bed of silica gel. The solvent was removed under reduced pressure to yield the reduced product (85-90%). The reduced diols can be further purified by recrystallization from hexanes:ethyl acetate for analytical purposes.

### Experimentals

#### TDO-catalyzed dihydroxylation of arenes

Biooxidation of ( $\pm$ )-1-phenyl-1-ethanol (**339**) with *P. putida* F39/D. The culture was prepared according to the procedure of Hudlicky *et al.*<sup>436</sup> The culture was induced by adding 150–200 mg of toluene to the media and incubated



in a shaker for 2 hours. Cells were centrifuged (3000 rpm, 10 min) and re-suspended in the same nutrient broth (250 mL). The racemic phenylethanol (300 mg) was then added to the culture and the progress of the biotransformation was monitored by UV (266 nm) and TLC. After 48 h, cells were removed by centrifugation and the supernatant was extracted with EtOAc (5x 250 mL). The organic layers were combined, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo* to give (**138**) a 3:2 mixture of diastereomers **337a** and **337b** respectively according to GLC analysis on an optically active cyclodextrine column and the <sup>1</sup>H NMR spectrum of the resulting metabolites were in accord with the reported value in literature.<sup>333</sup>

**3-(1(*S*)-Hydroxyethyl)cyclohexa-3,5-diene-1(*S*),2(*R*)-diol (337a)**

was obtained from biooxidation of (*S*)-1-phenyl-1-ethanol (339a)

according to the procedure described for racemic 1-phenyl-1-ethanol with

*P. putida* F39/D to provide standard metabolites for monitoring the biotransformation of racemic phenylethan-1-ol. The conversion was monitored by UV spectroscopy ( $\lambda_{\text{max}} = 266$  nm). After the usual work up, the process provided an isolated yield of 1g/L of metabolite 337a.



**3-(1(*R*)-Hydroxyethyl)-cyclohexa-3,5-diene-1(*S*),2(*R*)-diol (337b)**

was obtained from biooxidation of (*S*)-1-phenyl-1-ethanol (339b)

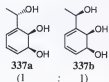
according to the procedure described for racemic 1-phenyl-1 ethanol with -

*P. putida* F39/D to provide a standard metabolite for monitoring the biotransformation of racemic phenylethan-1-ol. The conversion was monitored by UV spectroscopy ( $\lambda_{\text{max}} = 266$  nm) and after the usual work-up, the process provided an isolated yield of 1g/L of metabolite 337b.



**Biooxidation of ( $\pm$ )-1-phenyl-1-ethanol (339) with *E.***

*coli* JM109 (pDTG601). The cultures were prepared by using the procedure of Hudlicky *et al.*<sup>436,437</sup> The progress of



the biotransformation was monitored by UV ( $\lambda_{\text{max}} = 266$  nm). GLC analysis of the metabolites formed showed that the process gave rise to a 1:1 mixture of diastereomers 337a and 337b. <sup>1</sup>H NMR spectra of the metabolites were in agreement with the literature

values.<sup>333</sup> The biooxidation of racemic 1-phenyl-1-ethanol was also carried out on a large scale and gave the same 1:1 mixture of **337a** and **337b**.

**3-(Hydroxyethyl)cyclohexa-3-ene-(1S, 2R)-diol (340)** was made by treating the crude dienetriol **337** (1.50 g, 9.6 mmol) obtained from biooxidation of corresponding phenylethanol with PAD in the usual manner as described in the general procedure to afford the title compound as a colorless oil (1.4 g, 92%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.00 (bt, *J* = 3.5, 1H), 5.89 (bt, *J* = 3.7, 1H), 4.47–4.42 (m, 1H), 4.40–4.26 (m, 4H), 4.17–3.90 (m, 5H), 3.76–3.52 (m, 2H), 2.30–2.12 (m, 2H), 2.11–1.90 (m, 2H), 1.85–1.56 (m, 4H), 1.36–1.20 (m, 6H); <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>) δ 140.3, 139.2, 126.5, 124.9, 71.0, 69.6, 69.2, 69.1, 67.6, 65.8, 24.9, 24.7, 23.9, 23.4, 21.8, 21.6.



**(1S,2R)-3-(1-Cyclohexenyl)-3,5-cyclohexadien-1,2-diol (346)** was obtained from biotransformation of 1-phenylcyclohexene (**345**) with the *E. coli* JM109 (pDTG601) according to the general procedure for the large scale fermentation. Addition of 1-phenylcyclohexene



(15.7 g, 99.2 mmol) afforded a crude yield of 18.4 g (3 g/L) of **346** as an off-white solid.

$\lambda_{\text{max}}$  = 302 nm; mp 90–93 °C;  $[\alpha]_{\text{D}}^{28}$  +47.5 (c 0.5, MeOH); IR (KBr) 3226, 3050, 1435 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.16 (bt, *J* = 4.2, 1H), 6.01–5.89 (m, 2H), 5.75 (bd, *J* = 9.5, 1H), 4.49–4.43 (m, 1H), 4.34 (bd, *J* = 5.5, 1H), 2.70 (bs, 2H), 2.27–2.14 (m, 4H), 1.79–1.55 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 138.8, 133.8, 130.7, 125.7, 124.0, 117.9, 71.4, 66.7, 26.0, 25.3, 22.6, 22.0; MS(Cl): *m/z* 193 (1), 191 (27), 175 (100), 174 (79), 173 (22); HRMS (Cl) *m/z* calcd for C<sub>12</sub>H<sub>17</sub>O<sub>2</sub> (M+H): 193.1228. Found: 193.1213.

### Synthesis of (1*S*,2*R*)-3-(1-Cyclohexenyl)-3-cyclohexene-1,2-diol

(**352**). Into a flame-dried 50 mL round bottom flask equipped with a 3-way stopper,  $(\text{CH}_3\text{CN})_3\text{PdCl}_2$  (5 mg, 0.019 mmol) was added together with 5 mL of anhydrous DMF. A solution of cyclohexanone enol



triflate<sup>339</sup> (216 mg, 0.94 mmol) in dry DMF (5 mL) was added to the reaction flask via a syringe under argon. Trimethyl tin derivative of the vinyl bromide **348**<sup>338</sup> (296 mg, 0.94 mmol) was dissolved in dry DMF (5 mL) and the solution was added drop wise to the reaction flask via a syringe. The starting material was consumed after 4 h of stirring at room temperature according to TLC. The reaction mixture was washed with 5%  $\text{NaHCO}_3$  (2 x 10 mL), and the aqueous layer was extracted several times with pentane. The organic layers were combined, dried with  $\text{Na}_2\text{SO}_4$ , and the solvent was removed under vacuum to afford a red oil. The residue was dissolved in a solvent mixture of THF:H<sub>2</sub>O:TFA (4:1:1; 10 mL), and stirred for 1 h. THF and TFA were removed under vacuum and the residue was washed with 5%  $\text{NaHCO}_3$  (2x 5 mL) and water (2x 5 mL). The organic layer was dried  $\text{Na}_2\text{SO}_4$  and the product was purified by flash chromatography (1:4 hexane-EtOAc) to afford **352** as an off-white solid 43 mg (18%).  $R_f$  = 0.54; mp = 143–144 °C;  $[\alpha]_D^{28}$  -88.8 (c 1.0,  $\text{CHCl}_3$ ); IR (KBr) 3222, 3060, 1451  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  5.99 (m, 1H), 5.64 (m, 1H), 4.41 (d,  $J$  = 6.1, 1H), 4.24 (d,  $J$  = 5.2, 1H), 4.12 (m, 1H), 3.44–3.33 (m, 1H), 2.19–1.98 (m, 6H), 1.75–1.38 (m, 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  137.6, 134.3, 124.3, 123.5, 70.0, 65.9, 25.84, 25.77, 24.94, 24.85, 22.87, 22.21; MS(FAB)  $m/z$ : 194 (5), 177(22), 93(100); HRMS (CI)  $m/z$  calcd for  $\text{C}_{12}\text{H}_{18}\text{O}_2$  ( $M^+$ ): 194.1307. Found: 194.1253.

The title compound was also made via diimide reduction of the metabolite **346** obtained from biotransformation of 1-phenylcyclohexene **345**.

(1*S*,2*R*)-3-(1-Cyclohexyl)-3,5-cyclohexadien-1,2-diol (**354**) was generated via biooxidation of phenylcyclohexane **353** according to the general procedure for the large scale biotransformation. Addition of phenylcyclohexane (26.5 g; 165 mmol) over three hours during the fermentation process afforded 14.0 g (1.8 g/L) of **354** as an off-white solid.  $R_f = 0.32$  (1:1 hexane:EtOAc); mp = 63–65°C;  $\lambda_{\max} = 268$  nm;  $[\alpha]_D^{30} -73.8$  (c 1.0, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr) 3312, 3048, 1646, 1588, 1447; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.80 (ddd,  $J = 2.3, 5.4, 9.5$ , 1H), 5.62–5.56 (m, 2H), 4.67 (d,  $J = 6.4$ , OH), 4.30 (d,  $J = 6.3$ , OH), 4.08–4.00 (m, 1H), 3.75 (bt,  $J = 5.4$ , 1H), 2.10–1.98 (m, 1H), 1.86–1.60 (m, 5H), 1.34–0.96 (m, 5H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  146.9, 129.5, 123.0, 116.7, 69.7, 67.0, 41.4, 32.8, 31.2, 26.3, 26.2, 25.9.



**Correlation of absolute stereochemistry of metabolite 354.** The *cis*-diene diol **354** was reduced with PAD according to the general procedure. Purification by flash chromatography (hexane-EtOAc 1:4) afforded 0.98 g (86%) of **356** as a crystalline compound.

(1*S*,2*R*)-3-(1-cyclohexyl)-3-cyclohexene-1,2-diol (**356**).  $R_f = 0.35$  (1:1 hexane:EtOAc); mp = 68–69 °C;  $[\alpha]_D^{28} -75.2$  (c 1.1, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr) 3330, 1448 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  5.34 (t,  $J = 3.5$ , 1H), 4.31 (d,  $J = 5.9$ , 1H, OH), 4.27 (d,  $J = 5.6$ , 1H, OH), 3.80 (t,  $J = 4.5$ , 1H), 3.45–3.33 (m, 1H), 2.12–1.85 (m, 3H), 1.82–1.52 (m, 6H), 1.49–1.39 (m, 1H), 1.32–0.90 (m, 5H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  143.8, 120.9, 69.4, 67.0, 41.5, 38.0, 31.6, 26.6, 26.4, 26.0, 24.9, 24.2; MS (CI)  $m/z$  195 (3, M-1), 179 (100), 161 (18), 93



(33); HRMS (CI)  $m/z$  calcd for  $C_{12}H_{19}O$  (M-OH): 179.1436. Found: 179.1436. Anal. calcd for  $C_{12}H_{20}O_2$ : C, 73.43; H, 10.27. Found: C, 73.46; H, 10.38.

This compound upon protection as an acetonide furnished a sample whose spectroscopic and physical data were in accord with synthetic **357**.

(2,2-dimethyl-3a,7a-dihydrobenzo[1,3]dioxol-4-yl)cyclohexane (**357**) was synthesized from a procedure adapted from a report by Tamura and Kochi.<sup>342</sup> In a 100 mL 3-neck round bottom flask, magnesium metal



(98 mg, 4 mmol) was covered with dry  $Et_2O$  (5 mL) and the solvent

degassed with ultrasound under argon atmosphere for 30 min. Then 7-bromo-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[1,3]-dioxole **348**,<sup>338</sup> (0.699 g, 3.0 mmol) was added together with dry  $Et_2O$  (5 mL) and left in the ultrasound bath for 1 h. The reaction mixture was then cooled down to  $-78^\circ C$  and with rigorous stirring  $Li_2CuCl_4$  (30 mL, 0.1 M) in  $Et_2O$  was added over 15 min. Stirring was continued for another 30 min and then cyclohexyltriflate<sup>341</sup> (390 mg, 2.0 mmol) in dry  $Et_2O$  (10 mL) was added. The reaction mixture was stirred for 3 hours.

Work up in the usual manner followed by chromatography (5% EtOAc in hexane) afforded 18 mg (38%) of the product.  $[\alpha]_D^{27} = +49.2$  (c 0.9,  $CHCl_3$ );  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  5.52 (t,  $J = 3.0$ , 1 H), 4.39 (d,  $J = 5.6$ , 1H), 4.22-4.17 (m, 1H), 2.18-2.04 (m, 1H), 2.02-1.93 (bt,  $J = 11.3$ , 1H), 1.87-1.54 (m, 7H), 1.35 (s, 6H), 1.34-0.93 (m, 6H);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$  141.2, 122.9, 107.9, 73.6, 72.9, 40.8, 33.3, 31.5, 28.0, 26.8, 26.6, 26.5, 26.4, 25.6, 20.7; MS (FAB)  $m/z$  236 (0.6), 207 (40), 191 (21), 177 (12), 161 (18), 153 (42), 147 (100), 133 (42), 105 (21), 95 (35), 91 (63), 83 (70); HRMS (FAB)  $m/z$  calcd for  $C_{15}H_{24}O_2$ : 236.1776. Found: 236.1774.

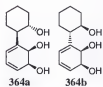
(1*S*,2*R*)-3-(2-Cyclohexenyl)-3,5-cyclohexadien-1,2-diol (**360**) was made according to the general procedure for the small scale biotransformation. Addition of 3-phenylcyclohexene<sup>438</sup> **359** (500 mg) afforded a crude yield of 120 mg of **360** as a reddish oil.  $\lambda_{\text{max}}$  = 262;



$R_f$  = 0.53 (2:3/hexane:EtOAc); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  5.86-5.75 (m, 4H), 5.66-5.51 (m, 6H), 4.64 (d, *J* = 5.97, 2H), 4.41 (d, *J* = 6.36, 2H), 4.05 (bs, 2H), 3.78 (t, *J* = 5.97, 2H), 2.95 (bs, 2H), 1.98-1.91 (m, 4H), 1.86-1.70 (m, 2H), 1.60-1.40 (m, 6H).

**Correlation of absolute stereochemistry of (360).** The crude metabolites (7.47 g, 38.9 mmol) from a large scale biotransformation of **359** were subjected to PAD reduction according to the general procedure to give rise to a mixture of inseparable diols **361** and **362**. The crude mixture of products **361** and **362** were protected as acetonides and subjected to catalytic hydrogenation in MeOH at 30 PSI with Wilkinson's catalyst. The product was isolated and purified by flash chromatography to afford a colorless oil (2.12g) whose spectroscopic data and physical data ( $[\alpha]_D^{28} +52.8$  (c 0.6, CHCl<sub>3</sub>)) were in accord with synthetic **357** and thus proved the absolute stereochemistry of **360**.

3-(2-hydroxycyclohexenyl)-3,5-cyclohexadien-1,2-diol (**364a** and **364b**) were obtained from biooxidation of ( $\pm$ )-*trans*-2-phenyl-1-cyclohexanol (**363**) according to the general procedure for large scale fermentation. Into a 15-L fermentor

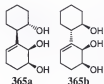


of JM109 (pDTG601) cells ( $OD_{640 \text{ nm}} = 45$ ), ( $\pm$ )-*trans*-2-phenyl-1-cyclohexanol **363** (10.29 g) was added (2 g every 30 min). The progress of the biotransformation was monitored by UV absorbance ( $\lambda=270 \text{ nm}$ ). Three hours after the first addition, the absorbance at 270 nm

ceased to increase. The pH of the medium was adjusted to 7.8 with concentrated  $\text{NH}_4\text{OH}$ , cells were removed by centrifugation and the supernatant was extracted with base-washed ethyl acetate to afford 10.25g (1.3 g/L) of a gray solid as a 1:1 mixture of inseparable diastereomers of 3-(2-hydroxycyclohexanyl)-3,5-cyclohexadien-1,2-diol (**364a** and **364b**).  $^1\text{H}$  (300MHz,  $\text{DMSO}-d_6$ )  $\delta$  5.87-5.75 (m, 2H), 5.75-5.68 (m, 2H), 5.65-5.55 (m, 2H), 4.77 (d,  $J=6.59$ , 1H), 4.74-4.65 (m, 2H), 4.59 (d,  $J=5.43$ , 1H), 4.53-4.47 (m, 1H), 4.29 (d,  $J=4.1$ , 1H), 4.18-4.08 (m, 2H), 3.84 (t,  $J=5.42$ , 1H), 3.72 (t, 1H), 3.40-3.23 (m, 1H), 2.04-1.80 (m, 4H), 1.77-1.54 (m, 6H), 1.42-1.06 (m, 9H).  $^{13}\text{C}$  (75MHz,  $\text{CDCl}_3$ )  $\delta$  143.8, 141.7, 129.7, 128.1, 124.4, 123.6, 123.5, 119.4, 75.6, 71.2, 71.1, 70.7, 69.2, 66.1, 52.9, 48.6, 35.2, 34.1, 32.2, 31.5, 25.7, 25.5, 25.0, 24.8.

#### Bicyclohexyl-6-ene-2,3,2'-triol (**365a** and **365b**).

Crude mixture products **364a** and **364b** (5.034 g, 23.94 mmol) obtained from biooxidation of ( $\pm$ )-*trans*-2-phenyl-1-cyclohexanol (**363**) was dissolved in 20 mL of cold MeOH.



The reaction flask was placed in an ice bath. With stirring, potassium axodicarboxylate (6.933 g, 35.67 mmol) was added. The solution of AcOH (9.53 mL, 166.46 mmol) in MeOH (10 mL) was dropwise added to the reaction mixture. The reaction was allowed to proceed overnight. Saturated  $\text{NaHCO}_3$  was added until effervescence ceased and the mixture was evaporated to remove MeOH and the residue was diluted with water (10 mL). The aqueous solution was extracted with EtOAc (4x30 mL) and dried with  $\text{MgSO}_4$ . The concentrated EtOAc residue was purified by flash-chromatography (4:1/EtOAc:Hexanes). The eluent was concentrated to afford a yellow oil (1.2284g, 24%).  $R_f=0.68$  (EtOAc),  $[\alpha]_D^{28}=-67.8$  ( $c=1.0$   $\text{CHCl}_3$ ).  $^1\text{H}$  (300MHz,  $\text{CDCl}_3$ )  $\delta$  5.51 (m, 1H), 5.45 (m, 1H), 4.84 (d,  $J=4.39$ , 1H), 4.75 (d,

$J=3.42$ , 1H), 4.56 (d,  $J=4.88$ , 1H), 4.37 (d,  $J=5.86$ , 1H), 4.31 (d,  $J=6.1$ , 1H), 4.25 (d,  $J=4.15$ , 1H), 3.88 (m, 1H), 3.73 (t,  $J=3.90$ , 1H), 3.48-3.28 (m, 3H), 3.27-3.15 (m, 1H), 2.14-1.44 (m, 4H), 1.94-1.78 (m, 3H), 1.78-1.40 (m, 11H), 1.40-1.02 (m, 8H).  $^{13}\text{C}$  ( $\text{CDCl}_3$  – 75MHz)  $\delta$ : 139.9, 138.7, 129.1, 125.5, 75.0, 71.8, 70.5, 70.0, 69.5, 65.6, 53.9, 50.0, 35.3, 34.2, 32.3, 32.0, 25.9, 25.7, 25.0, 24.8, 24.4, 24.3. IR ( $\text{CHCl}_3/\text{cm}^{-1}$ )  $\nu$ : 3355.8, 3017, 2933, 2858, 1449. MS( $\text{CI}^+$ ):  $m/z$  213 (29%), 195 (100%), 117 (46%), 99 (51%). HRMS: calcd for  $\text{C}_{12}\text{H}_{21}\text{O}_3$ , 213.1490. Found 213.1490.

**Bicyclohexyl-4,6-diene-2,3,2'-triol (364a)** was obtained from biooxidation of **(1S,2R)-(+)-trans-2-phenyl-1-cyclohexanol (363a)** with *E. coli* JM109 (pDTG601) as described for the small scale transformation of racemic 1-phenyl-1-ethanol. The progress of the biotransformations



was monitored by UV absorbance ( $\lambda_{\text{max}} = 270\text{--}272$  nm) and TLC (EtOAc). The metabolite was isolated according to the method described for ( $\pm$ )-1-phenyl-1-ethanol. The crude metabolite was immediately subjected to PAD reduction. Purification by flash chromatography (1:4 hexane-EtOAc) afforded **365a** (100 mg, 49%).

**(1S,2R,1'R,2'S)-3-(2'-Hydroxycyclohexanyl)-3-cyclohexene-1,2-diol (365a)**.  $R_f = 0.68$  (EtOAc); mp = 89–91°C;  $[\alpha]_{\text{D}}^{28} -82.3$  (c 0.6,  $\text{CH}_2\text{Cl}_2$ ); IR (film) 3329, 1447  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  5.50 (m, 1H), 4.83 (d,  $J = 4.5$ , 1H), 4.73 (d,  $J = 3.4$ , 1H), 4.30 (d,  $J = 6.1$ ,



1H), 3.73 (t,  $J = 3.9$ , 1H), 3.46-3.38 (m, 1H), 3.26-3.15 (m, 1H), 2.1-1.0 (m, 13H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  139.9, 125.5, 75.0, 69.9, 69.5, 49.9, 35.3, 32.3, 25.9, 25.1, 24.8, 24.2; MS( $\text{CI}^+$ ):  $m/z$  213 (22), 195 (100), 177 (87), 159 (29), 147 (59), 105 (24); HRMS:

calcd for  $C_{12}H_{21}O_3$ , 213.1490. Found 213.1456. Anal. calcd for  $C_{12}H_{20}O_3$ : C, 67.89; H, 9.50. Found: C, 67.60; H, 9.55.

**Bicyclohexyl-4,6-diene-2,3,2'-triol (364b)** was obtained from biooxidation of **(1R,2S)-(+)-trans-2-phenyl-1-cyclohexanol (363b)** with *E. coli* JM109 (pDTG601) as described for the small scale transformation of racemic 1-phenyl-1-ethanol. The progress of the biotransformations of racemic 1-phenyl-1-ethanol. The progress of the biotransformations was monitored by UV absorbance ( $\lambda_{max}$  = 270 nm) and TLC (EtOAc). The crude metabolite was reduced with PAD and purified by flash chromatography (1:4 hexane-EtOAc) to afford 102 mg (50%) of **365b**.

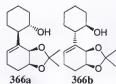
**(1S, 2R, 1'S, 2'R)-3-(2-hydroxycyclohexanyl)-3-cyclohexene-1,2-diol (365b)**.  $R_f$  = 0.68 (EtOAc); mp = 103–106°C;  $[\alpha]_D^{31}$  -148.0 (c 1,  $CH_2Cl_2$ ); IR (film) 3364, 1448  $cm^{-1}$ ;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  5.70 (dd,  $J$  = 2.6, 4.5 Hz, 1H), 4.16 (d,  $J$  = 3.3, 1H), 3.70–3.60 (m, 2H), 3.10 (bs, 1H), 2.85 (bs, 1H), 2.30–2.00 (m, 5H), 1.90–1.62 (m, 4H), 1.58–1.50 (m, 1H), 1.41–1.20 (m, 4H);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$  138.9, 129.5, 72.1, 70.7, 65.8, 54.1, 34.5, 32.2, 25.9, 25.3, 25.2, 24.7; MS(CI+):  $m/z$  213 (29), 195 (78), 177 (100), 159 (28), 147 (16), 105 (14); HRMS: calcd for  $C_{12}H_{21}O_3$ , 213.1490. Found: 213.1511. Anal. calcd for  $C_{12}H_{20}O_3$ : C, 67.89; H, 9.50. Found: C, 67.78; H, 9.62.

**Correlation of absolute stereochemistry of 364a and 364b.** The crude mixture from the biotransformation of ( $\pm$ )-**363** was reduced with PAD to afford **365a** and **365b**. Protection of the mixture of the *vic-cis*-diols **365a** and **365b** (392 mg, 2.0 mmol) were



carried out in neat dimethoxypropane with catalytic amount of *p*-toluenesulfonic acid yields the corresponding ketals **366a** and **366b**. The crude mixture (378 mg, 1.5 mmol) was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (10 mL) at 0 °C and  $\text{CBr}_4$  (746 mg, 2.25 mmol) was added followed by  $\text{PPh}_3$  (393 mg, 2.25 mmol) portion wise over 10-15 min. The mixture was left for another 2 h at ambient temperature. Chromatography (2.5% EtOAc in hexane) yielded the two diastereomeric bromides (441 mg, 90%) which were not characterized but were reduced in the general manner using  $\text{Bu}_3\text{SnH}$  and AIBN in dry, refluxing THF affording crude **357** (377 mg, 80%). Purification by chromatography (EtOAc:hexane 1:9) yielded an analytical sample with all spectroscopic and physical data in accord with **357** obtained from the synthetic approach described below.  $[\alpha]_D^{28} + 49.6$  (c 0.5,  $\text{CHCl}_3$ ).

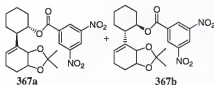
**2-(2,2-Dimethyl-3a,6,7,7a-tetrahydro-benzo[1,3]dioxol-4-yl)-cyclohexanol (366a)** and **2-(2,2-Dimethyl-3a,6,7,7a-tetrahydro-benzo[1,3]dioxol-4-yl)-cyclohexanol (366b)**. The triol mixture of **365a** and **365b**



(117 mg, 0.550 mmol) was dissolved in dimethoxypropane (DMP) (5 mL). To this mixture a catalytic amount of *p*TsOH was added and the reaction was monitored by TLC. After 30 min, the DMP solvent was removed under reduced pressure and the products were purified by flash chromatography (4:1 hexane:ethyl acetate) to afford the **366a** and **366b** alcohols mixture as a yellow oil (110 mg, yield 79%).  $R_f = 0.79$  (2:3 hexane:ethyl acetate).  $^1\text{H}$  NMR (acetone)  $\delta$  5.74 (m, 2 H), 4.23 (m, 2H), 4.24 (m, 2H), 2.20-2.03 (m, 2H), 2.06-2.01 (h, 2 H) 2.0-1.79 (m, 6 H), 1.78-1.53 (m, 10 H), 1.56-1.36 (m, 2 H), 1.34-1.11 (m, 20 H);  $^{13}\text{C}$  NMR (Acetone  $d_6$ )  $\delta$ : 138.46, 137.7, 130.3, 127.3, 108.3, 108.1,

74.2, 73.9, 73.86, 773.7, 72.2, 71.7, 54, 50.6, 35.2, 34.0, 32.77, 32.0, 28.0, 27.9, 26.67, 26.28, 26.25, 26.0, 26.0, 25.98, 25.25, 24.88, 21.28, 21.0; IR (CDCl<sub>3</sub>)  $\nu$ : 3462.1, 2983, 2856, 1448, 1377; Anal. calcd. for C<sub>12</sub>H<sub>24</sub>O<sub>3</sub>: C 71.39, H 9.59; Found C 71.37, H 9.76.

**3,5-Dinitro-benzoic acid 2-(2,2-dimethyl-3a,6,7,7a-tetrahydro-benzo[1,3]dioxol-4-yl)-cyclohexyl ester (367a) and 3,5-Dinitro-**



**benzoic acid 2-(2,2-dimethyl-3a,6,7,7a-tetrahydro-benzo[1,3]dioxol-4-yl)-cyclohexyl ester (367b).** Into a round-bottomed flask, alcohol mixture **366a** and **366b** (160 mg,

0.634 mmol) was dissolved in pyridine (5 mL) at ambient temperature. 3,5-

Dinitrobenzylchloride (95 mg, 0.413 mmol) was then added and the progress of the reaction was monitored via TLC. After 1 h of stirring at room temperature, the reaction mixture was diluted with EtOAc (20 mL) and washed with 5%, NaHCO<sub>3</sub> (10 mL, 2X), 5% CuSO<sub>4</sub> (10 mL, 3X). The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure.

The 3,5-dinitrobenzoate derivatives **367a** and **367b** were purified via recrystallization (EtOH) to give (190 mg, 67%) white solid, mp= 125-130 °C;  $R_f$  = 0.54 (4:1 hexane-EtOAc),  $[\alpha]_D^{27}$  = +17.6 (c 0.5, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H (CDCl<sub>3</sub>)  $\delta$  9.20 (q,  $J$  = 2.2, 1 H), 9.11 (d,  $J$  = 2.2, 1 H), 9.08 (d,  $J$  = 2.2, 1 H), 5.93 (t,  $J$  = 4.1, 1 H), 5.71 (t,  $J$  = 3.5, 1 H), 5.25 (ddd,  $J$  = 4.2, 10.5, 14.9, 1 H), 5.05 (ddd,  $J$  = 4.5, 10.8, 15.2, 1 H), 4.45 (t,  $J$  = 6.1, 2 H), 4.28 – 4.16 (m, 2 H), 2.48 – 1.22 (m, 24 H), 1.37 (s, 3 H), 1.33 (s, 3 H), 1.26 (s, 3 H), 1.10 (s, 3 H); <sup>13</sup>C (CDCl<sub>3</sub>)  $\delta$  162.2, 162.1, 148.7, 136.9, 136.5, 135.3, 134.6, 129.6, 129.4, 128.7, 126.8, 122.4, 122.1, 108.3, 108.2, 79.6, 77.4, 76.6, 73.8, 73.6, 72.9, 72.3, 49.3, 48.1, 32.5, 32.4, 32.2, 31.8, 28.1, 28.1, 26.5, 26.4, 26.1, 26.0, 25.7, 25.7, 24.8, 24.7, 21.5, 20.9; IR (CDCl<sub>3</sub>) 3102, 1726, 1628, 1600, 1547;

MS(Cl<sup>+</sup>)  $m/z$  446(2), 445 (9), 389 (16), 195 (20), 177 (100), 159 (81), 91 (97); HRMS: calcd. for C<sub>22</sub>H<sub>27</sub>N<sub>2</sub>O<sub>8</sub> (M+H) 447.1767, found 447.1764; Anal. Calcd. for C<sub>22</sub>H<sub>26</sub>N<sub>2</sub>O<sub>8</sub>: C 59.19, H 5.87, N 6.27; Found C 59.30, H 5.92, N 6.27.

(*R*)- and (*S*)-2-Phenylcyclohexanone (**368a** and **368b**) were made by oxidizing the corresponding optically active *trans*-2-phenylcyclohexanol with pyridinium chlorochromate (PCC) in CH<sub>2</sub>Cl<sub>2</sub> at 5 °C with the usual work up.

(*R*)-2-Phenylcyclohexanone (**368a**):  $R_f$  = 0.30 (Hexane:Et<sub>2</sub>O 4:1); mp 48-51 °C;  $[\alpha]_D^{27}$  +108.7 (c 0.20, benzene), lit.<sup>439</sup>  $[\alpha]_D^{26}$  +114.7 (c 0.45, benzene); IR (KBr) 3090, 1699 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.34 - 7.16 (m, 5H), 3.62 (dd,  $J$  = 12.0, 5.5 Hz, 2H), 2.55-2.38 (m, 2H), 2.27-2.22 (m, 1H), 2.14-2.08 (m, 1H) 2.02-1.95 (m, 2H), 1.85-1.77 (m, 2H); <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  210.2, 138.8, 128.8, 128.5, 126.7, 57.7, 42.0, 35.3, 27.6, 25.0.



(*S*)-2-Phenylcyclohexanone (**368b**):  $[\alpha]_D^{27}$  -109.8 (c 0.30, benzene), lit.<sup>439</sup>  $[\alpha]_D^{24}$  -113.5 (c 0.60, benzene); IR (KBr) 3092, 1695 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.36 -7.15 (m, 5H), 3.62 (dd,  $J$  = 12.0, 5.5 Hz, 2H), 2.56-2.39 (m, 2H), 2.28-2.24 (m, 1H), 2.13-2.08 (m, 1H) 2.02-1.95 (m, 2H), 1.85-1.78 (m, 2H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  210.2, 138.8, 128.8, 128.5, 126.7, 57.7, 42.0, 35.3, 27.6, 25.0.



**Biooxidations of ( $\pm$ )-2-phenylcyclohexanone (**368**) with *E. coli* JM109 (pDTG601)** were carried out as described for the small scale transformation for racemic

1-phenyl-1-ethanol. This afforded the acetals **369a** and **369b** together with the ketone **369c** as a 2:1:1 mixture, respectively.

The proof of structures and the correlation of absolute stereochemistry was provided by transforming the two enantiomers **368a** and **368b** separately as for racemic **368**.

(4*S*,4*aR*,9*aR*)-4,6,7,8,9*a*-Hexahydro-4*aH*-dibenzofuran-4,5*a*-diol (**369a**).  $\lambda_{\max}$  = 266 nm;  $R_f$  = 0.58 (1:1 hexane:EtOAc);  $[\alpha]_D^{28}$  +130.3 (c 0.7, MeOH); IR (film) 3378, 1449  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  6.09 (dd,  $J$  = 4.8, 9.4, 1H), 5.98 (dd,  $J$  = 4.3, 9.4, 1H), 5.84–5.78 (m, 1H), 4.76–4.70 (m, 1H), 4.25 (bs, 1H), 4.12 (bs, 1H), 3.97 (t,  $J$  = 5.5, 1H), 2.47–2.38 (m, 1H), 2.18–2.06 (m, 1H), 1.83–1.74 (m, 1H), 1.62–1.56 (m, 3H), 1.48–1.08 (m, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  144.3, 127.8, 124.2, 114.9, 106.9, 79.7, 61.6, 49.1, 33.9, 30.2, 24.0, 22.8; MS (CI):  $m/z$  192 (12), 191 (40), 132 (32), 128 (35), 119 (32), 107 (31), 105 (29), 91 (100), 83 (21), 81 (37); HRMS (CI)  $m/z$  calcd for  $\text{C}_{12}\text{H}_{13}\text{O}_2$  (M-OH): 191.1072. Found: 191.1090.



369a

(1*S*,5'*S*,6'*R*)-5',6'-Dihydroxybicyclohexyl-1',3'-dien-2-one (**369b**).  $R_f$  = 0.42 (1:1 hexane:EtOAc);  $[\alpha]_D^{28}$  +58.1 (c 0.6, MeOH); IR (film) 3352, 1702, 1444  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  6.06 (dd,  $J$  = 4.7, 9.5, 1H), 5.95 (dd,  $J$  = 5.6, 9.5, 1H), 5.81–5.77 (m, 1H), 4.74–4.69 (m, 1H), 4.20 (bs, 2H), 3.95 (t,  $J$  = 5.6, 1H), 2.45–2.38 (m, 1H), 2.14–2.04 (m, 1H), 1.80–1.72 (m, 1H), 1.68–1.58 (m, 3H), 1.46–1.12 (m, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  213.2, 139.2, 129.2, 124.0, 114.6, 68.7, 68.5, 42.1, 30.1, 27.3, 25.9, 22.8; HRMS (CI)  $m/z$  calcd for  $\text{C}_{12}\text{H}_{17}\text{O}_3$  (M+H): 209.2675. Found: 209.2669.



369b

(4*S*,4*aR*,9*aS*)-4,6,7,8,9,9*a*-Hexahydro-4*aH*-dibenzofuran-4,5*a*-diol (**369c**).  $\lambda_{\text{max}}$  = 266 nm;  $R_f$  = 0.49 (1:1 hexane:EtOAc);  $[\alpha]_D^{28}$  - 126.3

(c 0.5, MeOH); IR (film) 3371, 1454  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$

6.05 (dd,  $J$  = 4.8, 9.4, 1H), 5.92 (dd,  $J$  = 4.3, 9.4, 1H), 5.80-5.75 (m, 1H),

4.72-4.68 (m, 1H), 4.20 (bs, 1H), 4.10 (bs, 1H), 3.98 (t,  $J$  = 5.5, 1H), 2.48-2.40 (m, 1H),

2.17-2.07 (m, 1H), 1.85-1.77 (m, 1H), 1.60-1.54 (m, 3H), 1.45-1.10 (m, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ) 144.8, 127.8, 124.4, 114.4, 105.7, 79.7, 61.7, 49.0, 33.8, 30.1, 24.2, 22.7;

HRMS (CI)  $m/z$  calcd for  $\text{C}_{12}\text{H}_{13}\text{O}_2$  (M-OH): 191.1072. Found: 191.1081.



**Correlation of absolute stereochemistry of 369a, 369b, and 369c.** The crude mixture from the biotransformation of ( $\pm$ )-**368** was reduced with  $\text{NaBH}_4$  in MeOH to afford a mixture of diastereomers of alcohols **370a** and **370b**. This product mixture was reduced with PAD according to the general procedure and the biochemically installed *cis*-diol was protected as a ketal using dimethoxypropane and catalytic amount of *p*-toluenesulfonic acid. The crude mixture (756 mg, 3 mmol) was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (25 mL) at  $0^\circ\text{C}$  and  $\text{CBr}_4$  (1.49 g, 4.5 mmol) was added, followed by portion wise addition of  $\text{PPh}_3$  (786 mg, 4.5 mmol) over 15 min. The mixture was left for another two hours at ambient temperature. Chromatography (2.5% EtOAc in hexane) yielded 702 mg (85%) of the two diastereomeric bromides, which were reduced in the general manner using  $\text{Bu}_3\text{SnH}$  and AIBN in dry, refluxing THF, affording crude **357** (589 mg, 72 %). Purification by chromatography (EtOAc:hexane 1:9) yielded an analytical sample and all spectroscopic and physical data were in accord with synthetic **357**, ( $[\alpha]_D^{28}$  + 50.1 (c 0.6,  $\text{CHCl}_3$ )).

(1S, 2R)-3-benzyl-3,5-cyclohexadien-1,2-diol (**373**) was

obtained from biotransformation of diphenylmethane (**372**) according



to the general procedure for large scale biotransformation. To 8L of

fully grown (OD = 65) cells, diphenylmethane (17 g, 101 mmol) was slowly added over a

period of 3 h, and the progress of the biotransformation was monitored by UV ( $\lambda_{\text{max}} = 266$  nm). After the usual workup including extractions and concentration, the process gave (8.8

g, 1.1 g/L) metabolite **373** as an off-white solid.  $R_f = 0.47$  (2:3 Hexane:EtOAc); mp = 69–71

°C;  $[\alpha]_D^{33} + 117$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ); IR (film) 3440, 3054, 2987, 1643, 1422, 1265, 896, 745,

705;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  7.34–7.26 (m, 2H), 7.23–7.16 (m, 3H), 5.85–5.75 (m,

1H), 5.68–5.60 (m, 1H), 5.54 (d,  $J = 4.43$ , 1H), 4.64 (d,  $J = 6.36$ , 1H), 4.52 (d,  $J = 6.17$ , 1H),

3.70 (t,  $J = 6.16$ , 1H), 3.46 (s, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  141.3, 139.1, 129.2, 128.7,

128.2, 126.6, 124.9, 121.1, 69.9, 69.3, 40.3; MS(FAB)  $m/z$ : 202 (12), 185 (56), 184 (14),

107 (14), 93 (31), 91(100); HRMS (CI)  $m/z$  calcd for  $\text{C}_{13}\text{H}_{14}\text{O}_2$ : 202.0994. Found: 202.0997.

**Correlation of absolute stereochemistry of (1S, 2R)-3-benzyl-3,5-cyclohexadien-1,2-diol (**373**).** Into a flame-dried one-necked 25 mL round bottom flask equipped with a 3-way stopper, vinyl bromide **348**<sup>338</sup> (172 mg, 0.746 mmol) was dissolved in freshly distilled THF (10 mL). The reaction vessel was cooled to  $-78^\circ\text{C}$ , upon which *s*-BuLi (0.86 mL, 1.12 mmol) was added dropwise via syringe. The reaction was allowed to stir at  $-78^\circ\text{C}$  for 45 minutes. Benzyl bromide (0.191 g, 1.12 mmol) was then added and the reaction was allowed to proceed for one hour at  $-78^\circ\text{C}$  and then allowed to slowly warm to room temperature. The reaction was then quenched with 10%  $\text{NH}_4\text{Cl}$  (5 mL). The organic layer was diluted with ethyl acetate (10 mL). The layers were separated and the organic layer was washed with DI water (10 mL, 2X), dry with  $\text{MgSO}_4$ . The solvent was removed under vacuum to afford a crude yield of 123 mg (68%) as light yellow oil. The crude product was dissolved in 6 mL of

a solvent mixture 4:1:1 THF:H<sub>2</sub>O:TFA and allowed to stir at room temperature for one hour. Organic solvent was then removed under vacuum and the residue was diluted with ethyl acetate (10 mL), washed with 5% NaHCO<sub>3</sub> (5 mL, 2X). The organic layers were combined, dry with Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The product was purified by flash chromatography to afford a white solid (84 mg, 82%). Physical and spectroscopic data for this compound obtained from this route matched with the PAD reduced diol **374** obtained from the biooxidation, and thus proved the absolute stereochemistry of **373**.

**(1S, 2R)-3-Benzyl-cyclohex-3-ene-1,2-diol (374)**. An aliquot of the crude extract (2 g, 9.9 mmol) from biooxidation of substrate **372** was subjected to PAD (5.76 g, 29.7 mmol) reduction according to the general procedure. The product was purified by chromatography (1:1 hexane:EtOAc) to afford a white solid (0.98 g, 49%). mp = 73.5-75°C; [ $\alpha$ ]<sub>D</sub><sup>26</sup> -160.4 (c 1.0, MeOH); IR (KBr) 3281, 3024, 1602, 1494, 1453, 1260, 1078 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  7.32-7.23 (m, 2H), 7.21-7.12 (m, 3H), 5.36 (s, 1H), 4.47 (d, *J* = 5.7, 1H), 4.31 (dd, *J* = 5.7, 1H), 3.62 (t, *J* = 4.5, 1H), 3.44-3.31 (m, 3H), 2.11-2.08 (m, 2H), 1.70-1.52 (m, 1H), 1.50-1.38 (m, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  139.7, 137.4, 129.2, 128.6, 127.2, 126.4, 69.8, 68.2, 41.0, 25.5, 24.1; MS(FAB) *m/z*: 188 (12), 187 (100), 185 (26), 169 (19), 93 (31), 91(38); HRMS (CI) *m/z* calcd for C<sub>13</sub>H<sub>16</sub>O<sub>2</sub>: (M+OH): 187.1123. Found: 187.1125. Anal. calcd for C<sub>13</sub>H<sub>16</sub>O<sub>2</sub>: C, 76.44; H, 7.90. Found: C, 76.20; H, 7.93.



### Cyclopropylbenzene project

**3-Cyclopropylcyclohexa-3,5-diene-1,2-diol (383)** was isolated from the crude mixture collected from the biotransformation of cyclopropylbenzene. This was accomplished by centrifugation of the cells and extraction



of the broth with base-washed ethyl acetate. This process provides essentially pure dienediol which can be further purified by flash-chromatography (Hexanes:EtOAc / 4:1) to afford a white solid;  $R_f=0.4$  (hexanes:EtOAc / 1:1); mp=45-46 °C (decomposition);  $[\alpha]_D^{28} +11.39^\circ$  (c 1.75, CD<sub>3</sub>OD); IR (film) 3274, 3008, 2911, 1453, 1090, 992, 804 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, Acetone-d<sub>6</sub>)  $\delta$  5.75-5.85 (m, 1H), 5.54-5.66 (m, 2H), 4.17-4.26 (m, 1H), 3.63-3.81 (m, 2H), 3.40-3.51 (d,  $J=7$  Hz, 1H), 1.48-1.62 (m, 1H), 0.40-0.79 (m, 4H); <sup>13</sup>C NMR (75 MHz, Acetone-d<sub>6</sub>)  $\delta$  130.2, 127.4, 124.7, 118.7, 71.2, 69.0, 15.8, 7.6, 6.6.

**2-Cyclopropylphenol** was isolated as byproduct from the biotransformation of cyclopropyl benzene. The phenol was purified by distillation to afford a clear oil;  $R_f = 0.42$  (hexane:EtOAc / 8:1); IR (film)



3536, 1609, 1580, 1491, 752 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  9.26 (s, 1H), 6.86-6.92 (m, 1H), 6.63-6.76 (m, 3H), 2.02-2.12 (m, 1H), 0.80-0.87 (m, 2H), 0.55-0.61 (m, 2H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  155.8, 129.4, 125.9, 124.5, 119.0, 114.6, 9.3, 7.7; MS(EI)  $m/z$ : 136 (23), 134 (100), 133 (24), 115 (27), 107 (39), 106 (21), 105 (28), 91 (34), 89 (41), 78 (24), 77 (96), 61 (43); HRMS (EI)  $m/z$  calc'd for C<sub>9</sub>H<sub>10</sub>O (M<sup>+</sup>): 134.0732. Found: 134.0732.

**7-Cyclopropyl-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[1,3]dioxole (386).** To a well-stirred solution of diol **387** (0.150 g, 1.0 mmol) in 2,2-dimethoxypropane (DMP) (20 mL), a catalytic amount of *p*-toluenesulfonic acid was added. The reaction mixture was stirred for 30



minutes then was quenched with 5% NaHCO<sub>3</sub> (2 mL). Solvent was removed under reduced pressure. The residue was diluted with brine (20 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 30

mL). The organic layers were combined, dried ( $\text{MgSO}_4$ ), and concentrated under reduced pressure. The crude residue was purified by flash chromatography (8:1 Hexane:EtOAc) to provide **386** (0.151 g, 80%).  $R_f = 0.57$  (Hexane:EtOAc / 8:1);  $[\alpha]_D^{20} +58.99^\circ$  (c 1.0,  $\text{CH}_3\text{OH}$ ); IR (film) 3082, 2985, 1456, 1367, 870  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.51 (t,  $J = 3.91$  Hz, 1H), 4.37 (d,  $J = 4$  Hz, 1H), 4.25-4.31 (m, 1H), 2.09-2.22 (m, 1H), 1.75-1.96 (m, 2H), 1.60-1.72 (m, 2H), 1.42 (d,  $J = 5$  Hz, 6H), 0.32-0.71 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  137.3, 123.4, 108.5, 74.5, 73.8, 28.2, 26.8, 25.9, 20.9, 14.6, 5.9, 5.1; MS(EI)  $m/z$ : 179 (57), 137 (33), 136 (66), 121 (29), 108 (25), 107 (28), 95 (21), 91 (100), 79 (45), 77 (23), 55 (26); HRMS (EI)  $m/z$  calc'd for  $\text{C}_{12}\text{H}_{18}\text{O}_2$  ( $\text{M}^+$ ): 194.1307. Found: 194.1317.

### 3-cyclopropylcyclohex-3-ene-1,2-diol (**387**):

A) From diol **383**: To a well-stirred and ice-cooled solution of diol **383** (17 g, 0.11 mol) in methanol (50 mL), potassium azadicarboxylate (PAD) (76 g, 0.35 mol), was added. A solution of acetic acid (64 mL, 1 mol) in methanol (50 mL) was added dropwise to the reaction mixture at such a rate that produced 1-2 bubbles per second. The mixture was allowed to warm to ambient temperature, with the color changing from yellow to milky white. The reaction mixture was stirred overnight and then quenched with saturated  $\text{NaHCO}_3$  until effervescence ceased. Methanol was removed under reduced pressure. The residue was diluted with brine (20 mL), and extracted with ethyl acetate (4 x 40 mL). The combined organic phases were dried ( $\text{MgSO}_4$ ) and concentrated to yield a reddish oil. The crude product was purified by flash silica gel chromatography (3:2 Hexane:Ethylacetate) to afford **387** (12.411 g, 73%); as a white solid:  $R_f = 0.20$  (Hexane:EtOAc / 3:2); mp = 96-98  $^\circ\text{C}$ ;  $[\alpha]_D^{25} -126.0^\circ$  (c 1.0,  $\text{CH}_3\text{OH}$ ); IR (film) 3272, 3012, 1461, 989  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  5.32 (s, 1H), 4.32-4.36 (m, 1H), 3.65 (t,  $J$



= 4Hz, 1H), 3.35-3.42 (m, 2H), 1.92-1.99 (m, 2H), 1.52-1.63 (m, 1H), 1.38-1.46 (m, 2H), 0.33-0.56 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  138.6, 123.4, 70.0, 68.9, 25.3, 24.2, 14.9, 5.6; MS(FAB)  $m/z$ : 137(8), 93(100); HRMS (FAB)  $m/z$  calc'd for  $\text{C}_9\text{H}_{13}\text{O}_1$  ( $\text{MH}^+ - \text{H}_2\text{O}$ ): 137.0966. Found: 137.0967; Anal. calc'd for  $\text{C}_9\text{H}_{14}\text{O}_2$ : C, 70.1; H, 9.15. Found C, 70.17; H, 9.18.

**B) From vinyl bromide 348:** A 50 mL round bottom flask equipped with a condenser and an addition funnel under argon atmosphere was charged with magnesium metal (0.76 g, 31.2 mmol) and a crystal of iodine. The addition funnel was charged with a solution of cyclopropyl bromide (0.76 g, 6.24 mmol) in anhydrous diethyl ether (10 mL) and this solution was added dropwise into the reaction flask. Once the reaction proceeded on its own, the addition was continued to maintain a constant reflux. After the addition was completed the reaction flask was heated to reflux for an additional 45 minutes. The Grignard reagent **384** was then transferred into another reaction flask containing bromoacetone **348** (1.29 g, 5.61 mmol) and tetrakis(triphenylphosphine) palladium (0.194 g, 0.17 mmol) in THF (25 mL). The reaction was refluxed for 5 hours and the reaction mixture was filtered through a thin layer of silica. The filtrate was concentrated under reduced pressure. Flash chromatography provided the coupled product **386** (0.174 g, 16%). The acetone **386** was deprotected using a 4:1:1/THF:H<sub>2</sub>O:TFA (10 mL) solvent mixture affording diol **387** (0.104 g, 75%), whose physical data matched the compound obtained via the biotransformation of cyclopropylbenzene followed by PAD reduction.

**Acetic acid 6-acetoxy-2-cyclopropylcyclohex-2-enyl ester (388):** To a well-stirred solution of diol **387** (0.105 g, 0.68 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL), pyridine (0.16 g, 2.0 mmol) was added. Addition of acetic anhydride (0.21



g, 2.0 mmol) was followed by dimethylaminopyridine catalyst (0.2 mg, 0.20 mol%). The reaction mixture was allowed to stir for 20 hours and quenched with saturated citric acid (2 mL). The reaction mixture was diluted with brine (20 mL) extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 30 mL). The organic layers were combined, dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated under reduced pressure. The crude residue was purified by flash silica gel chromatography (3:2 Hexane:EtOAc) to afford **388** (0.156 g, 96%) as a white solid:  $R_f = 0.79$  (Hexane:EtOAc / 3:2); mp = 80.5-81.5 °C;  $[\alpha]_D^{29} -196.9^\circ$  (c 1.0,  $\text{CH}_3\text{OH}$ ); IR (film) 1739, 1460, 1248  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.82 (t,  $J = 3.2$  Hz, 1H), 5.52 (d,  $J = 3.2$  Hz, 1H), 4.92-4.99 (m, 1H), 2.14-2.22 (m, 2H), 2.10 (s, 3H), 2.01 (s, 3H), 1.84-1.91 (m, 1H), 1.70-1.74 (m, 1H), 1.22-1.28 (m, 1H), 0.50-0.64 (m, 2H), 0.30-0.47 (m, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  170.9, 170.6, 135.6, 125.5, 70.8, 68.4, 24.3, 22.6, 21.3, 21.2, 14.8, 5.6, 5.0; MS (EI)  $m/z$ : 179 (100), 119 (92), 91 (24); HRMS (EI)  $m/z$  calc'd for  $\text{C}_{13}\text{H}_{19}\text{O}_4$  ( $\text{MH}^+$ ): 239.1283. Found: 239.1289. Anal. calc'd for  $\text{C}_{13}\text{H}_{18}\text{O}_4$ : C, 65.63; H, 7.61. Found C, 65.43; H, 7.60.

### Substituted Catechol Project

3-methylcatechol (**98**) was obtained through biooxidation of toluene with *E. coli* JM109 (pDTG602) as described in the general procedure for preparation of catechols. The catechol was purified by Kugelrohr distillation



(90-95°C/2- 3mm Hg) yielding 19g (2.0g/L) of a white solid; mp 45-46 °C (lit.<sup>41</sup> 55-56); IR (KBr) 3422, 1624  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  9.04 (s, OH, 1H), 8.06 (s, OH, 1H), 6.64-6.55, (m, 1H), 6.53-6.44 (m, 2H), 2.10 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  143.2, 142.2, 124.8, 123.2, 120.4, 113.2, 15.6; MS (FAB)  $m/z$ : 93 (85), 124 (100); HRMS (CI)  $m/z$  calc'd for  $\text{C}_7\text{H}_8\text{O}_2$  ( $\text{M}^+$ ): 124.0524. Found: 124.0590.

**3-Bromocatechol (270)** was obtained through biooxidation of bromobenzene with *E. coli* JM109 (pDTG602), as described in the general procedure for preparation of catechols. The catechol 3-bromocatechol was



purified by distillation using Kugelrohr apparatus (80-90°C/2mmHg) to yield 9g (1g/L) of white solid; mp 39-40°C (lit.<sup>388</sup> 40.5-41.5); IR (KBr) 3484, 1592; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 9.76 (s, OH, 1H), 9.07 (s, OH, 1H), 6.90 (dd, *J* = 1.5, 8.1, 1H), 6.76 (dd, *J* = 1.5, 7.9, 1H), 6.57 (dd, *J* = 7.9, 8.1, 1H); <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>) δ 146.4, 142.9, 122.7, 120.1, 114.5, 109.8; MS(FAB) *m/z*: 190 (6), 188 (6), 137 (60), 136 (70), 107 (33), 95 (50), 81 (63); HRMS (CI) *m/z* calcd for C<sub>6</sub>H<sub>5</sub>BrO<sub>2</sub> (M<sup>+</sup>): 187.9473. Found: 187.9469; Anal. calcd for C<sub>6</sub>H<sub>5</sub>BrO<sub>2</sub>: C, 38.13; H, 2.67. Found: C, 38.14; H, 2.69.

**3-Fluorocatechol (277)** was obtained through biooxidation of fluorobenzene with *E. coli* JM109 (pDTG602) that was carried out as described in the general procedure for preparation of catechols. The



catechol 3-fluorocatechol was purified by distillation using Kugelrohr apparatus (80-90°C/0.2mmHg) to yield 12g (1.3g/L) of white solid; mp 69-70 °C; IR (KBr); <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 9.44 (s, OH, 1H), 8.97 (s, OH, 1H), 6.62-6.52 (m, 3H); <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>) δ ; HRMS (FAB) *m/z* calcd for C<sub>6</sub>H<sub>5</sub>O<sub>2</sub>F (M<sup>+</sup>): 128.0273. Found: 128.0275; Anal. calcd for C<sub>6</sub>H<sub>5</sub>O<sub>2</sub>F: C, 56.26; H, 3.93. Found: C, 56.26; H, 4.05.

**3-Methoxycatechol (279)** was obtained through biooxidation anisole with *E. coli* JM109 (pDTG602) as described in the general procedure for preparation of catechols. The metabolite was purified by flash-



chromatography to yield white powder, mp 35-37°C,  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  6.75 (t,  $J = 8.3$ , 1H), 6.60 (dd,  $J = 1.3$ , 8.3, 1H), 6.46 (dd,  $J = 1.3$ , 8.3, 1H), 3.89 (s, 3H), 1.52 (bs, 2H);  $^{13}\text{C}$  NMR (75MHz,  $\text{CDCl}_3$ )  $\delta$  146.9, 144.0, 132.4, 119.7, 108.7, 103.0, 56.1.

**3-Chlorocatechol (398)** was obtained through biooxidation of chlorobenzene with *E. coli* JM109 (pDTG602) that was carried out as described in the general procedure for preparation of catechols, yielding a concentration of 0.5 g/L; mp 46-48 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  9.69 (s, OH, 1H),



9.05 (s, OH, 1H), 6.76 (dd,  $J = 1.7$ , 8.1, 1H), 6.71 (dd,  $J = 1.7$ , 8.1, 1H), 6.61 (t,  $J = 8.1$ , 1H).

**3-Isopropyl-6-methylcatechol (400)** was obtained through biooxidation of *p*-cymene with *E. coli* JM109 (pDTG602) as described in the general procedure for preparation of catechols. The catechol was purified by Kugelrohr (75-80 °C /2 mmHg) distillation yielding 3.6g (0.4g/L) of the title compound; mp 47-48 °C (lit. 47.5-48); IR (KBr) 3447, 1629, 1578  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300



MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.09 (s, OH, 1H), 7.97 (s, OH, 1H), 6.51 (d,  $J = 8.1$ , 1H), 6.49 (d,  $J = 8.1$ , 1H), 3.16 (heptet,  $J = 6.8$ , 1H), 2.09 (s, 3H), 1.11 (d,  $J = 6.8$ , 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  141.6, 141.1, 132.7, 122.1, 121.4, 117.6, 27.2, 22.8 (x2), 15.5; MS(FAB)  $m/z$ : 166 (100), 151 (51), 125 (30), 93 (75); HRMS (CI)  $m/z$  calcd. for  $\text{C}_{10}\text{H}_{14}\text{O}_2$  ( $\text{M}^+$ ): 166.0993. Found: 166.0985. Anal. calcd for  $\text{C}_{10}\text{H}_{14}\text{O}_2$ : C, 72.26; H, 8.49. Found: C, 72.37; H, 8.61.

**3-Iodocatechol (402)** was obtained through biooxidation of iodobenzene with *E. coli* JM109 (pDTG602) as described in the general procedure for preparation of catechols. The catechol was purified by



procedure for preparation of catechols. The catechol was purified by Kugelrohr distillation (80-90 °C/2mmHg) to yield 4.5 g (0.5 g/L) of white solid; mp 55-56 °C (lit.<sup>1</sup> 55-56); <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 9.74 (s, OH, 1H), 9.13 (s, OH, 1H), 7.08 (dd, *J* = 1.5, 7.9, 1H), 6.76 (dd, *J* = 1.5, 7.9, 1H), 6.42 (t, *J* = 7.9, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 143.6, 143.0, 129.5, 123.2, 116.0, 84.5; MS(FAB) *m/z*: 236 (11), 209 (18), 185 (80), 93 (100); HRMS (CI) *m/z* calcd for C<sub>6</sub>H<sub>5</sub>IO<sub>2</sub> (M<sup>+</sup>): 235.9334. Found: 235.9250. Anal. calcd for C<sub>6</sub>H<sub>5</sub>IO<sub>2</sub>: C, 30.53; H, 2.14. Found: C, 30.66; H, 2.19.

**3-Ethynyl-benzene-1,2-diol (403)** was obtained through biooxidation of ethynylbenzene with *E. coli* JM109 (pDTG602) as described in the general procedure for preparation of catechols. The metabolite was purified by flash-chromatography (3:2 hexane:EtOAc) to yield white crystalline compound (0.75g/L). *R<sub>f</sub>* = 0.35 (3:2 hexane:EtOAc); mp = 57.5-59.5 °C; IR (film) 3490, 3284, 2101, 1617, 1586, 1469, 1332, 1266, 1200, 1064, 955, 831, 780, 673; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.00-6.91 (m, 2H), 6.85-6.75 (m, 1H), 5.60 (bs, 2H), 3.45 (s, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 144.9, 143.9, 123.7, 121.2, 117.0, 108.6, 84.3, 78.3; MS (CI); *m/z* 134 [M<sup>+</sup>] (100), 78 (88), 77 (87), 74 (23), 63 (18) 62 (33), 52 (47), 51 (97), 50 (73); Anal. Calcd for C<sub>8</sub>H<sub>6</sub>O<sub>2</sub>: C, 71.64; H, 4.51. Found: C, 71.71, H, 4.56.



**3-Cyclopropylbenzene-1,2-diol (404)** was prepared by biotransformation of the corresponding arene with *E. coli* JM109 (pDTG602). *R<sub>f</sub>* = 0.52 (Hexane:EtOAc / 3:2); mp = 41-42 °C; IR (film) 3418, 1622, 1592, 1477, 775 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 9.11 (s, 1H), 8.11 (s, 1H), 6.50-6.58 (m, 1H), 6.49 (t, *J* = 7.82 Hz, 1H), 6.16-6.21 (m, 1H), 2.01-2.12 (m, 1H),



0.79-0.86 (m, 2H), 0.50-0.57 (m, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  143.4, 142.9, 128.8, 120.6, 119.7, 113.5, 9.4, 6.1; MS(EI)  $m/z$ : 150 (100), 149 (6), 107 (26), 77 (44); HRMS (EI)  $m/z$  calc'd for  $\text{C}_9\text{H}_{10}\text{O}_2$  ( $\text{M}^+$ ): 150.0681. Found: 150.0675. Anal. calc'd for  $\text{C}_9\text{H}_{10}\text{O}_2$ : C, 71.98, H, 6.71. Found: C, 72.07, H, 6.69

**6-Chloro-3-methoxycatechol (405)** was obtained through biooxidation of 4-chloroanisole with *E. coli* JM109 (pDTG602) as described in the general procedure for preparation of catechols, the metabolite was purified by flash-chromatography (4:1 Hexane:EtOAc). The fractions were combined and concentrated under reduced pressure. Pentane was added to afford 6-chloro-3-methoxycatechol as white precipitate (4.0g, 0.5g/L).  $R_f = 0.37$  (3:2 hexane:EtOAc); mp = 115-116 °C; IR (KBr) 3379, 3081, 3059, 3025, 1600, 1492, 1451, 756, 700;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  9.14 (s, 1H), 8.94 (s, 1H), 6.56-6.53 (m, 1H), 6.51-6.48 (m, 1H), 3.75 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  146.2, 140.2, 133.8, 119.6, 113.3, 103.7, 56.5; MS(CI):  $m/z$  176 (33), 174 (100), 155 (27), 154 (88), 138 (38) 137 (56), 136 (53) 107 (21), 91 (13), 83 (13), 81 (12); Anal. calcd for  $\text{C}_7\text{H}_7\text{ClO}_3$ : C, 48.16; H, 4.04. Found: C, 48.13; H, 4.01.



**1-Bromo-2,3-dihydroxy-4-methoxybenzene (406)** was obtained through biooxidation of 4-bromoanisole with *E. coli* JM109 (pDTG602) as described in the general procedure for preparation of catechols, the metabolite was purified by flash-chromatography (1:1 Hexane:EtOAc) and recrystallized from octanes:pentane solvent mixture to afford white crystalline 1-bromo-2,3-dihydroxy-4-methoxybenzene:  $R_f = 0.58$  (1:1 hexane:EtOAc); mp = 121-122 °C; IR (KBr) 3442, 3054, 1486, 1434, 754, 688;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  9.15 (s, 1H), 8.98 (s, 1H), 6.87 (d,



$J=8.9$ , 1H), 6.45 (d,  $J=8.9$ , 1H), 3.76 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  149.0, 144.4, 136.2, 123.3, 105.8, 102.7, 57.1; MS(Cl):  $m/z$  218 (7), 209 (7), 185 (100), 93 (122); Anal. calcd for  $\text{C}_7\text{H}_7\text{BrO}_3$ : C, 38.38; H, 3.22. Found: C 38.49; H, 3.23.

**3-Methoxy-6-methylbenzene-1,2-diol (407)** was obtained through biooxidation of 4-methylanisole with *E. coli* JM109 (pDTG602) as described in the general procedure for preparation of catechols. The title metabolite was purified by flash chromatography (1:1 hexane:EtOAc) to yield 5.2 g (0.65 g/L) of white crystalline compound; mp 92-93.5 °C (lit. 92.5-93.5 °C);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  6.61 (d,  $J=8.3$ , 1H), 6.38 (d,  $J=8.3$ , 1H), 5.49 (bs, 2H), 3.84 (s, 3H), 2.20 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  145.2, 142.4, 132.3, 120.7, 117.9, 102.5, 56.3, 15.2.



**3-Cyclohexyl-benzene-1,2-diol (408)** was obtained through biooxidation of cyclohexylbenzene with *E. coli* JM109 (pDTG602) as described in the general procedure for preparation of catechols. The catechol was purified by flash chromatography (1:1 Hexane:EtOAc) and recrystallized from toluene:octane to afford 0.55 g (0.07 g/L) of a white solid.  $R_f = 0.68$  (3:2 /hexane:EtOAc); mp 139-140 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  9.13 (s, OH, 1H), 7.95 (s, OH, 1H), 6.63-6.51, (m, 3H), 2.96-2.72 (m, 1H), 1.88-1.62 (m, 5H), 1.44-1.12 (m, 5H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  143.0, 141.4, 134.5, 120.4, 119.2, 112.8, 37.6, 33.2, 27.1, 26.4.



**3-Phenoxy-pyrocatechol** was obtained through biooxidation of phenylether with *E. coli* JM109 (pDTG602), as described in the general procedure for preparation of catechols. The metabolite was purified by flash chromatograph.  $^1\text{H}$  (DMSO)  $\delta$  9.35 (s, 1 H), 8.64 (s, 1 H), 7.37-



7.24 (m, 2 H), 7.06-6.94 (m, 1 H), 6.88-6.78 (m, 2 H), 6.70-6.56 (m, 2H), 6.46-6.36 (m, 1 H)  $^{13}\text{C}$  ( $\text{CDCl}_3$ )  $\delta$ : 156.9, 145.3, 144.0, 135.0, 130.0, 123.8, 120.5, 118.2, 111.3, 110.9.

### Chemoenzymatic approach to combetastatin A-1 and B-1 project

**2-Methoxy-5-bromophenol** this metabolite was obtained from biotransformation of 4-bromoanisole. The product was purified by chromatography and the red residue was triturated with pentane to afford a



white precipitate: MP 64-65 (lit. 60-62 C), IR (KBr) 3400, 1592, 1262,  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  9.50 (s, OH, 1H), 6.93-6.83 (m, 3H), 3.73 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  146.7, 146.0, 123.0, 118.0, 113.4, 112.0, 56.3; MS (CI);  $m/z$  202 [ $\text{M}^+$ ] (100), 194 (42), 189 (40), 124 (41), 108 (64) 106 (142), 95 (22), 91 (174), 83 (120), 82 (35) 81 (197); HRMS (EI)  $m/z$  calcd for  $\text{C}_7\text{H}_5\text{BrO}_2$  ( $\text{M}^+$ ): 201.9629. Found: 201.9627. Anal. Calcd for  $\text{C}_7\text{H}_5\text{BrO}_2$ : C, 41.41; H, 3.48. Found: C, 41.67, H, 3.47.

### 1-Bromo-2,3-bis(methoxymethoxy)-4-methoxybenzene (436):

To a flame-dried round bottom flask fitted with a 3-way stopper, were added bromoanisole catechol **406** (2.34 g, 10.6 mmol) and  $\text{CH}_2\text{Cl}_2$

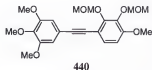


(15 mL) followed by  $\text{EtN}(\text{iPr})_2$  (5.49 g, 42.5 mmol) and then MOMCl (3.42 g, 42.5 mmol). The reaction mixture was allowed to stir at room temperature under argon

atmosphere for 3 hours. The reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (15 mL), washed with

NaHCO<sub>3</sub> (2X, 10 mL), then with water (2X, 10 mL). The organic layer was dried with MgSO<sub>4</sub> and the solvent was removed under reduced pressure. The resulting red oil was purified by flash-chromatography (9:1/pentane:acetone) to afford a colorless oil (2.96 g, 89%). *R*<sub>f</sub> = 0.33 (9:1 pentane:acetone); IR (film) 3087, 2961, 2835, 1577, 1477, 1390, 1292, 1221, 1159; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.25 (d, *J* = 8.9, 1H), 6.61 (d, *J* = 8.9, 1H), 5.2 (s, 2H), 5.13 (s, 2H), 3.82 (s, 3H), 3.66 (s, 3H), 3.60 (s, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 153.5, 148.4, 140.2, 127.7, 109.1, 108.9, 99.6, 98.9, 58.4, 57.7, 56.3; MS(FAB): *m/z*, 306 (M<sup>+</sup>, 35), 277 (61), 275 (66), 244 (55), 242 (57), 231 (77), 230 (100), 229 (82) 165 (26), 151 (33); HRMS (FAB) *m/z* calcd for C<sub>11</sub>H<sub>15</sub>BrO<sub>5</sub>: 306.0102. Found: 306.0157. Anal. calcd for C<sub>11</sub>H<sub>15</sub>BrO<sub>5</sub>: C, 43.02; H, 4.92. Found: C, 43.48; H, 4.92.

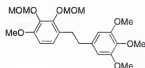
**3-Methoxy-6-[2-(3,4,5-trimethoxyphenyl)ethynyl]benzene-1,2-bis-methoxymethoxy-benzene (440).** 3,4,5-



trimethoxy-ethynylbenzene (**306**) (239 mg, 1.246 mmol) was dissolved in a 10:1 mixture of DME:THF (5 mL) under Argon. The mixture was cooled to -78 °C for 10 min. and *n*BuLi (1.33 mmol) was then added and allowed to stir at -78 °C for 45 min. Upon which time, B(OiPr)<sub>3</sub> (234 mg, 1.246 mmol) was added and the reaction was allowed to proceed for another hour. The reaction mixture was then transferred into another reaction vessel containing MOM-protected-bromoanisole catechol **436** (256 mg, 0.831 mmol) in 10:1/DME:THF (10 mL) along with Pd(PPh<sub>3</sub>)<sub>4</sub> (29 mg, 0.025 mmol) under refluxing conditions, with constant argon passing through. The reaction was allowed to proceed at reflux for another 4h. The catalyst was removed by filtration through a silica bed. The filtrate was concentrated and the product was purified by flash-chromatography to afford

yellowish powder compound (0.256 g, 73%). mp = 84–85 °C;  $R_f$  = 0.48 (3:2/hexane:EtOAc); IR (film);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.26 (s, 1H), 6.74 (s, 2H), 6.69 (d,  $J$  = 8.4, 1H), 5.31 (s, 2H), 5.14 (s, 2H), 3.87 (s, 3H), 3.86 (s, 9H), 3.66 (s, 3H), 3.61 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  154.5, 153.3, 151.9, 139.2, 139.1, 138.9, 128.8, 118.7, 111.0, 108.8, 108.0, 106.8, 99.4, 98.9, 92.2, 85.1, 68.5, 68.4, 61.1, 57.8, 57.6, 56.3, 56.2; FABMS (%):  $m/z$  418 (100)  $[\text{M}^+]$ , 387 (21), 343 (17), 342 (13), 154 (13), 137 (10), 95 (13), 83 (17), 81 (14); HRMS (FAB)  $m/z$  calcd for  $\text{C}_{22}\text{H}_{26}\text{O}_8$ : 418.1627. Found: 418.1620. Anal. calcd for  $\text{C}_{22}\text{H}_{26}\text{O}_8$ : C, 63.15; H, 6.26. Found: C, 63.14; H, 6.17.

**3-Methoxy-6-[2-(3,4,5-trimethoxy-phenyl)ethyl]benzene-1,2-bis-methoxymethoxy-benzene (441).** Acetylene **440** (72 mg) in MeOH

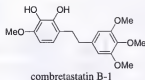


**441**

(10 mL) and 10% Pd/C (10 mg) was treated with a positive pressure of  $\text{H}_2$  (30 PSI) at ambient temperature overnight. The catalyst was removed by filtering through a bed of silica. The product was purified by flash chromatography to afford the title compound **441** (65 mg, 93%) as colorless oil.  $R_f$  = 0.46 (3:2/hexane:EtOAc); IR (film) 2939, 2839, 1589, 1495, 1458, 1277, 1126, 1065, 971, 804;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  6.83 (d,  $J$  = 8.54, 1H), 6.63 (d,  $J$  = 8.54, 1H), 6.40 (s, 2H), 5.15 (s, 2H), 5.12 (s, 2H), 3.83 (s, 3H), 3.82 (s, 9H), 3.60 (s, 3H), 3.58 (s, 3H), 2.88 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  153.1, 152.1, 149.6, 138.6, 138.0, 136.1, 128.1, 124.6, 107.7, 105.4, 99.8, 98.7, 61.0, 57.8, 57.5, 56.1, 56.1, 37.4, 32.1; FABMS(%):  $m/z$  422 (80)  $[\text{M}^+]$ , 391 (28), 359 (51), 345 (68), 287 (19), 219 (15), 194 (21), 191 (26), 181 (91), 179 (22), 167 (27), 165 (100), 155 (21), 154 (57), 137 (38), 128 (38), 109 (38), 97 (48), 95 (45), 91 (28), 83 (68), 81 (61); HRMS (FAB)  $m/z$  calcd for  $\text{C}_{22}\text{H}_{30}\text{O}_8$ : 422.1940. Found: 422.1951.

**Combretastatin B-1: Compound 441 (49**

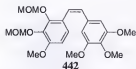
mg) was dissolved in a 1:1 mixture of 6N HCl:THF solvent (2 mL) and allowed to stir at ambient



temperature for 1h. The reaction mixture was diluted with H<sub>2</sub>O (10 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3X, 15 mL). Organic layers were combined, concentrated and the desired product was purified by flash chromatography to afford colorless oil (42 mg).  $R_f = 0.73$  (7:3/hexane:EtOAc), IR (film) 3428, 2937, 1629, 1590, 1509, 1487, 1420, 1288, 1239, 1126, 1093, 1006; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  6.57 (d,  $J = 8.28$ , 1H), 6.42 (s, 1H), 6.38 (d,  $J = 8.47$ , 1H), 5.41 (s, D<sub>2</sub>O exchangeable, 1H), 5.39 (s, D<sub>2</sub>O exchangeable, 1H), 3.85, (s, 3H), 3.83 (s, 6H), 3.82 (s, 3H), 2.93-2.79 (m, 4H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  153.1, 145.5, 142.2, 138.4, 136.1, 132.4, 121.6, 120.3, 105.5, 102.5, 61.1, 56.3, 56.2, 36.9, 32.1; MS(FAB):  $m/z$ , 335 (M<sup>+</sup>, 71), 334 (100), 307 (24), 289 (15), 181 (58), 167 (30), 155 (45), 154 (130), 138 (53), 128 (30), 120 (21), 111 (37), 109 (41), 107 (36), 105 (30); HRMS (FAB)  $m/z$  calcd for C<sub>18</sub>H<sub>23</sub>O<sub>8</sub>: 335.1494. Found: 335.1490.

**MOM-protected combretastatin A-1 (442).**

Borane THF (5 mL, of a 1 M solution in THF) was slowly added to distilled cyclohexene (1.02 mL, 10



mmol) in THF (5 mL) at 0 °C. This mixture was stirred for 1h at 0 °C, affording a dense white precipitate after 10 min. The alkyne 426 (100 mg, 0.24 mmol) in THF (5 mL) was added and the resulting yellow mixture was stirred for 1 h at 0 °C after which time the precipitate had dissolved giving a clear yellow solution. The solution was allowed to warm to ambient temperature and stirred for 30 min. HOAc (1.72 mL, 30 mmol) was added and the

solution stirred at 0 °C for 2 h. H<sub>2</sub>O (30 mL) was added and the mixture extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 25 mL). The organic extracts were washed with saturated NaHCO<sub>3</sub> (3X, 25 mL), H<sub>2</sub>O (30 mL), dried (MgSO<sub>4</sub>) and concentrate in vacuo. The product was purified by flash-chromatography affording the title compound as a red oil (80 mg, 80%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.91 (d, J= 8.79, 1H), 6.65 and 6.51 (AB system, J=12 Hz, 2H), 6.56 (d, J=8.79 Hz, 1H), 6.48 (s, 2H), 5.19 (s, 2H), 5.12 (s, 2H), 3.82 (s, 6H), 3.65 (s, 6H), 3.60 (s, 3H), 3.57 (s, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 153.2, 153.0, 149.3, 147.4, 139.0, 137.3, 132.7, 130.2, 126.1, 125.6, 125.3, 107.8, 106.4, 99.4, 98.8, 61.0, 57.7, 57.5, 56.2, 56.0.

**Combretastatin A-1.** Compound **442** (50 mg) was

dissolved in a 1:1 mixture of 3N HCl:THF solvent (5 mL)

and allowed to stir at ambient temperature for 3 hr. The

reaction mixture was diluted with H<sub>2</sub>O (10 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3X, 15 mL).

Organic layers were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), concentrated in vacuo, and the desired

product was purified by flash chromatography to afford the title compound as a white solid

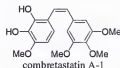
(30.6 mg, 77% yield). mp = 140-142 °C; R<sub>f</sub> = 0.42 (1:1/hexane:EtOAc), <sup>1</sup>H NMR (300 MHz,

CDCl<sub>3</sub>) δ 6.76 (d, J= 8.64, 1H), 6.59 and 6.53 (AB system, J=12 Hz, 2H), 6.52 (s, 2H), 6.38

(d, J=8.4, 1H), 5.41 (s, D<sub>2</sub>O exchangeable, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 3.67 (s, 6H); <sup>13</sup>C

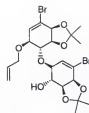
NMR (75 MHz, CDCl<sub>3</sub>) δ 153.0, 146.5, 141.8, 137.4, 132.8, 132.7, 130.5, 124.3, 120.6,

118.0, 106.1, 103.1, 61.1, 56.4, 56.1.



## Polyhydroxylated Chiral Polymer Project

5-(5-Allyloxy-7-bromo-2,2-dimethyl-3a,4,5,7a-tetrahydro-benzo[1,3]dioxol-4-yloxy)-7-bromo-2,2-dimethyl-3a,4,5,7a-tetrahydro-benzo[1,3]dioxol-4-ol (**450**). Into a flame-dried 50 mL RBF equipped with a stir bar under argon atmosphere, allylalcohol (0.56 g, 9.66 mmol) was dissolved in methylene

**450**

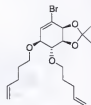
chloride (5 mL). Bromoepoxide **448** was then added and the reaction flask was cooled to  $-78^{\circ}\text{C}$  for 20 min.  $\text{BF}_3\cdot\text{Et}_2\text{O}$  was then added and the reaction was allowed to proceed over night. The reaction was quenched with water (10 mL) and extracted with methylene chloride (10 mL, 3X). The organic layers were combined and washed with 5%  $\text{NaHCO}_3$  (10 mL, 1X), dry ( $\text{MgSO}_4$ ) and evaporate to afford a reddish residue. The product was purified by flash-chromatography (4:1/Hexanes:EtOAc) to yield the title compound as a white solid (256 mg, 56%).  $R_f = 0.65$  (3:2/Hexanes:EtOAc); mp =  $176\text{--}177^{\circ}\text{C}$ ;  $[\alpha]_D^{31} = +47.35$  (c, 0.665,  $\text{CHCl}_3$ ); IR (KBr) 3498, 2981, 1645, 1457, 1381, 1214, 1046, 998, 870, 513;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  6.33 (d,  $J = 1.71$ , 1H), 6.28 (d,  $J = 1.71$ , 1H), 6.18–5.85 (m, 1H), 5.39–5.24 (m, 2H), 4.72 (d,  $J = 6.84$ , 1H), 4.47 (d,  $J = 6.84$ , 1H), 4.46 (s, 1H), 4.28–4.12 (m, 3H), 4.45–4.00 (m, 1H), 3.90 (d,  $J = 8.30$ , 1H), 3.86–3.80 (m, 1H), 3.67 (m, 1H), 3.53 (t,  $J = 8.7$ , 1H), 1.58 (s, 3H), 1.57 (s, 3H), 1.42 (s, 3H), 1.41 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  135.1, 133.5, 132.8, 118.8, 118.1, 117.9, 111.5, 111.1, 84.4, 84.1, 78.7, 77.6, 77.20, 77.1, 74.1, 74.0, 71.2, 28.2, 27.7, 25.9, 25.7. HRMS (FAB) calcd for  $\text{C}_{21}\text{H}_{29}\text{O}_7\text{Br}_2$ : 551.0280. Found: 551.0045. Anal. calcd for  $\text{C}_{21}\text{H}_{29}\text{O}_7\text{Br}_2$ : C, 45.67; H, 5.11. Found: C, 45.92; H, 5.28.

4,5-Bis-allyloxy-7-bromo-2,2-dimethyl-3a,4,5,7a-tetrahydro-benzo[1,3]dioxole (**451**). Into a 50 mL oven-dried round-bottomed flask, NaH (2.41 g, 45 mmol) was added under argon atmosphere and covered with anhydrous DMF (20 mL). The mixture was allowed to



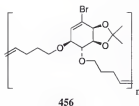
stir for 1h at room temperature under argon atmosphere after the addition of bromodiol **451** (2.41 g, 9.1 mmol). Allylbromide (5.46 g, 45 mmol) dissolved in 10 mL of DMF was then dropwise added to the reaction mixture and allowed to proceed for another 8h after the completion of allylbromide addition. The reaction was quenched with water and extracted with diethylether (25 mL, 3X). The product was purified by chromatograph (9:1/hexanes:EtOAc) and the fractions containing the titled compound were combined and concentrated to yield a yellowish oil, which was further purified by distillation at high temperature and under reduced pressure (150 °C, 0.2 mm Hg) using Kulgerolh apparatus to afford bis-allylether **452** as a colorless oil (3.14 g, 98%).  $R_f = 0.44$  (6:1/hexanes:EtOAc);  $[\alpha]_D^{27} = 36.85^\circ$  (c 1.1,  $\text{CDCl}_3$ ); IR (film) 3078, 2987, 1646, 1456, 1381, 1246, 1218, 1073, 869, 794  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  6.21 (d,  $J=2.19$ , 1H), 6.02-5.84 (m, 2H), 5.33 (dt,  $J=1.47$ , 1.71, 1H), 5.28 (dt,  $J=1.47$ , 1.71 Hz, 1H), 5.24-5.14 (m, 2H), 4.65 (dd,  $J=1.22$ , 6.35 Hz, 1H), 4.40-4.25 (m, 2H), 4.24-4.19 (dd,  $J=6.35$ , 8.55 Hz, 1H), 4.16 (t,  $J=1.46$  Hz, 1H), 4.14 (t,  $J=1.46$  Hz, 1H), 3.90-3.84 (ddd,  $J=1.22$ , 0.98, 7.82 Hz, 1H), 3.52 (t,  $J=8.05$  Hz, 1H), 1.55 (s, 3H), 1.41 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  135.2, 134.5, 133.6, 119.0, 117.4, 117.4, 110.8, 79.2, 78.0, 77.9, 77.65, 73.5, 71.7, 28.2, 26.3; HRMS (EI)  $m/z$  calc'd for  $\text{C}_{14}\text{H}_{18}\text{O}_4\text{Br}$  [M-CH<sub>3</sub>]<sup>+</sup>: 329.0388. Found: 329.0271. Anal. calc'd for  $\text{C}_{15}\text{H}_{21}\text{O}_4\text{Br}$ : C, 52.19; H, 6.13. Found C, 52.35; H, 6.23.

7-Bromo-2,2-dimethyl-4,5-bis-pent-4-enyloxy-3a,4,5,7a-tetrahydro-benzo[1,3]dioxole (**455**). A 50 mL oven-dried round-bottomed flask was charged with NaH (0.66 g, 22 mmol) under argon atmosphere at room temperature. Anhydrous DMF (10 mL) was then added, followed by bromodiol **451** (0.73 g, 2.75 mmol)

**455**

and the reaction mixture was allowed to stir for 1h at room temperature. 5-Bromopentene (3.28 g, 22 mmol) dissolved in DMF (10 mL) was then added drop-wise to the reaction mixture, which was allowed to stir for another 6h. The reaction was quenched with water (5 mL) and extracted with diethyl ether (25 mL, 3X). The ethereal fractions were combined, dried ( $\text{MgSO}_4$ ), and concentrated under reduced pressure to afford a reddish oil. The products were purified by flash-chromatography (9:1/Hexanes:EtOAc) and the fractions containing the titled compound were combined and concentrated. The resulting yellowish oil was distilled under reduced pressure (155°C, 0.2mmHg) using Kulgerolh apparatus to furnish the titled compound as a colorless oil (0.35g, 32%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  6.2 (d,  $J=3.05$  Hz), 5.92-5.74 (m, 2H), 5.05 ( $J=2.05$ , 1.54, 1H), 5.03-4.92 (m, 3H), 4.63 (dd,  $J=1.03$ , 6.41, 1H), 4.21-4.12 (dd,  $J=6.41$ , 8.46 Hz, 1H), 3.86-3.67 (m, 3H), 3.65-3.51 (m,  $J=2.56$ , 5.64, 6.41, 6.67 Hz, 2H), 3.39 (t,  $J=8.1$  Hz), 2.20-2.08 (m, 4H), 1.77-1.63 (m, 4H), 1.54 (s, 3H), 1.41 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  138.5, 138.2, 133.8, 118.8, 115.2, 114.8, 110.7, 80.0, 79.0, 78.0, 77.5, 72.3, 70.0, 30.4, 30.8, 29.5, 29.3, 28.3, 26.3; HRMS (EI)  $m/z$  calc'd for  $\text{C}_{19}\text{H}_{29}\text{O}_4\text{Br}$ : 400.1249 Found: 400.1256.

ADMET polymerization of vinylbromo ether **455** to polymer **456**. A 10 mL round-bottomed flask equipped with a magnetic stir bar, monomer **455** (1.68 g, 4.19 mmol) was charged and degassed through several freeze- pump-thaw cycles under high vacuum.



Grubbs' second-generation catalyst (35 mg, 1%) was then added under argon atmosphere. A few drops of dry  $\text{CDCl}_3$  were added on occasion in order to facilitate reaction initiation. After the addition of Grubbs' catalyst, very slow to moderate bubbling of ethylene was observed. The bubbling reaction was then exposed to intermittent vacuum until the viscosity increased, followed by exposure to high vacuum to remove ethylene, which is continuously generated during the course of the polymerization. The reaction was started and maintained at room temperature until the increase in viscosity prevented stirring. At this point, the reaction temperature was slowly raised to  $55^\circ\text{C}$  over a period of 3-5 days until a very high viscosity was obtained or bubbling ceased. The reaction mixture was then cooled to room temperature, and quenched by exposure to air. The viscous residue was dissolved in ethyl acetate and passed through a short column of silica to furnish a brown viscous oil (1.58 g).

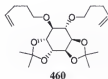
**2,2,7,7-Tetramethyl-5-pent-4-enyloxy-hexahydro-benzo[1,2-d;3,4-d']bis[1,3]dioxol-4-ol (459)**. The di-acetonide protected epoxide **458** (1.0 g, 4.13 mmol) was dissolved in freshly distilled methylene chloride (15 mL) along with the 4-penten-1-ol



(0.64 mL, 6.19 mmol) and cooled in an ice-bath for 15 minutes.  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (10 mol %) was then added and the reaction was allowed to proceed overnight. The reaction was quenched

with water and extracted with methylene chloride (25 mL, 3X). The organic layers were combined, dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated. The product was purified by flash-chromatography (5:1/hexanes:EtOAc) to yield the title compound (0.794 g, 59%) as a colorless semi-solid material.  $R_f$  = 0.25 (5:1/hexane:EtOAc),  $[\alpha]_D^{27}$   $-29.4^\circ$  (c 1.1,  $\text{CH}_3\text{OH}$ ); IR (film) 3459, 2986, 1380, 1260, 1067, 1025  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.92-5.74 (m, 1H), 5.10-4.92 (m, 2H), 4.28-4.12 (m, 4H), 3.96-3.84 (m, 1H), 3.70-3.50 (m, 2H), 3.30-3.20 (m, 1H), 2.93 (s, exchanged with  $\text{D}_2\text{O}$ , 1H), 2.22-2.10 (m, 2H), 1.80-1.65 (m, 2H), 1.51 (s, 6H), 1.36 (s, 3H), 1.34 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  138.42, 115.11, 110.3, 110.0, 80.0, 79.3, 78.5, 76.9, 76.8, 71.5, 70.9, 30.5, 29.2, 27.9, 27.8, 25.4, 25.2; HRMS (EI)  $m/z$  calc'd for  $\text{C}_{16}\text{H}_{25}\text{O}_6$   $[\text{M}-\text{CH}_3]^+$ : 313.1651. Found: 313.1649.

**2,2,7,7-Tetramethyl-4,5-bis-pent-4-enyloxy-hexahydro-benzo[1,2-d;3,4-d']bis[1,3]dioxole (460).** A 10 mL round-bottomed flask was charged with NaH (169 mg, 4.2 mmol) and covered with anhydrous DMF (5 mL). Alcohol



459 (138 mg, 0.42 mmol) dissolved in DMF (5 mL) was then added dropwise to the reaction flask and allowed to stir at room temperature for 45 min. 5-Bromopentene (0.62 mL, 4.2 mmol) was then added and the reaction was allowed to proceed for 5h. The reaction was quenched with  $\text{H}_2\text{O}$  (5 mL) and extracted with  $\text{Et}_2\text{O}$  (10 mL, 3X). The ethereal portions were combined, washed with saturated NaCl (10 mL),  $\text{H}_2\text{O}$  (10 mL), dried ( $\text{MgSO}_4$ ) and concentrated. The product was purified by flash-chromatography (9:1/Hexanes:EtOAc) to yield a thick red oil (96 mg, 93%).  $R_f$  = 0.68 (5:1/Hexane:EtOAc),  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.90-5.72 (m, 2H), 5.08-5.02 (m, 1H), 5.02-4.90 (m, 3H), 4.50-4.32 (m, 1H), 4.24-4.10 (m, 4H), 3.80-3.66 (m, 4H), 3.36-3.26 (m, 2H), 2.20-2.06 (m, 4H), 1.76-1.63 (m, 4H),

1.49 (s, 6H), 1.40 (s, 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  138.6, 114.8, 114.7, 112.3, 109.6, 80.3, 79.4, 77.3, 76.7, 75.6, 74.2, 71.7, 70.9, 30.4, 29.9, 29.5, 29.3, 28.0, 26.5, 25.6, 24.3.

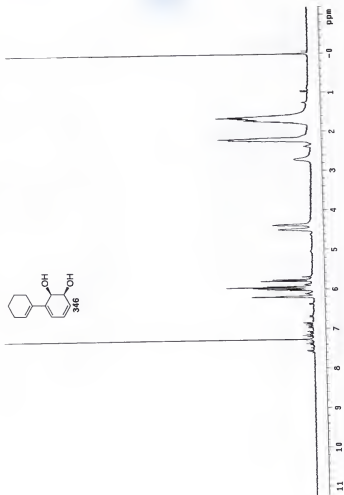
#### ADMET polymerization of diene **460** to polymer **461**.

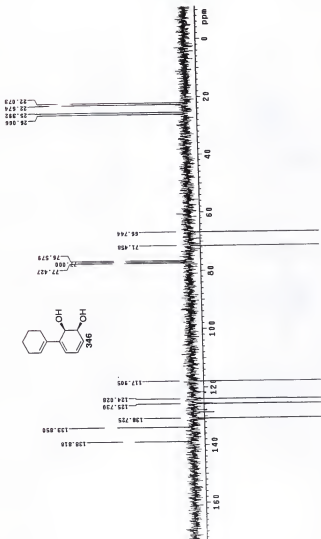
A 10 mL round-bottomed flask equipped with a magnetic stir bar, monomer **460** (240 mg, 0.61 mmol) was charged and degassed through several freeze-pump-thaw cycles under high vacuum.

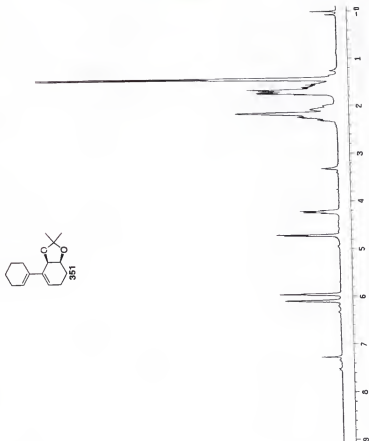


Grubbs' first generation catalyst (5 mg) was then added under argon atmosphere. After the addition of Grubbs' catalyst, very slow to moderate bubbling of ethylene was observed. The bubbling reaction was then exposed to intermittent vacuum until the viscosity increased, followed by exposure to high vacuum to remove ethylene, which is continuously generated during the course of the polymerization. The reaction was maintained at room temperature until the increase in viscosity prevented stirring. At this point, the reaction temperature was slowly raised to 45°C over a period of 3-5 days until bubbling ceased. The reaction mixture was then cooled to room temperature, and quenched by exposure to air. The viscous residue was dissolved in ethyl acetate and passed through a short column of silica to furnish a brown viscous oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.50-5.40 (b, 2H), 4.25-4.15 (b, 4H), 3.80-3.65 (m, 4H), 3.31 (s, 2H), 2.20-1.80 (b, 4H), 1.75-1.55 (m, 4H), 1.50 (s, 6H), 1.33 (s, 6H). The polymer **461** was dissolved in a solvent mixture of 4:1:1/THF:TFA:H<sub>2</sub>O (5 mL) to arrive at fully hydroxylated polymer **462**.

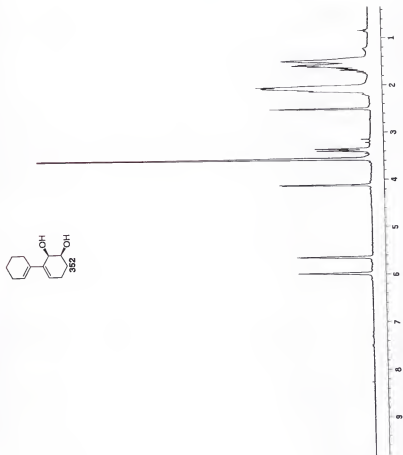
CHAPTER 6  
SELECTED SPECTRAL DATA

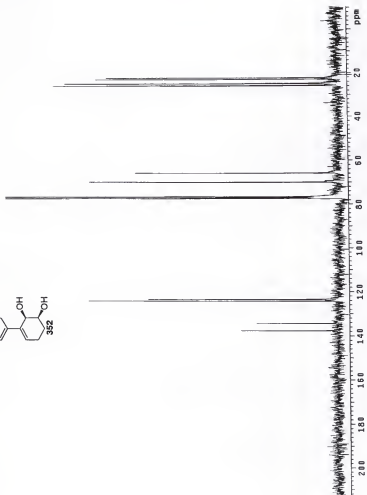


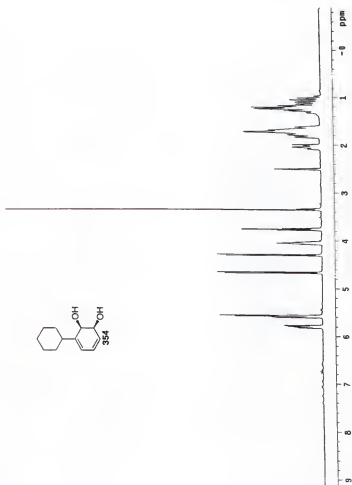


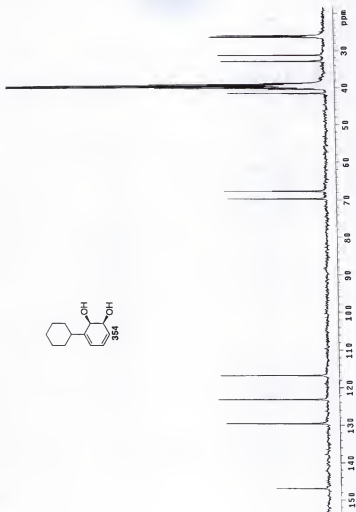


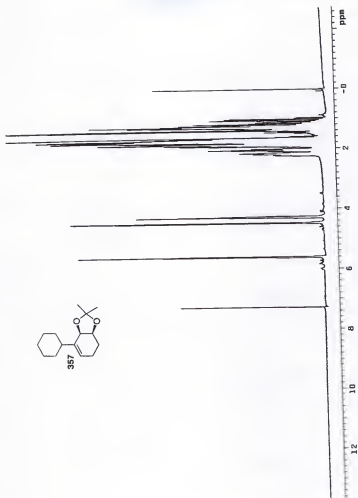


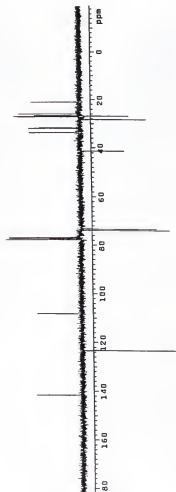


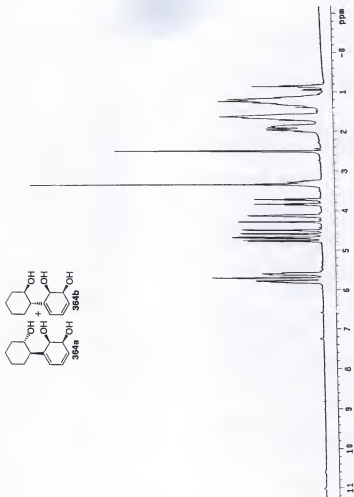


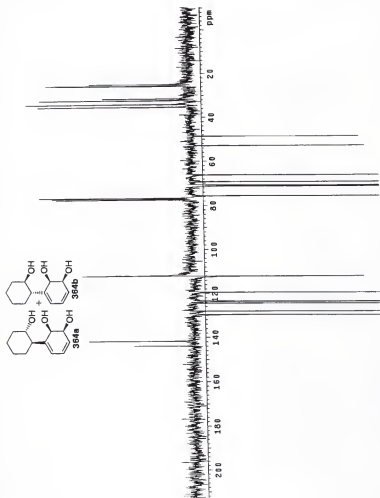


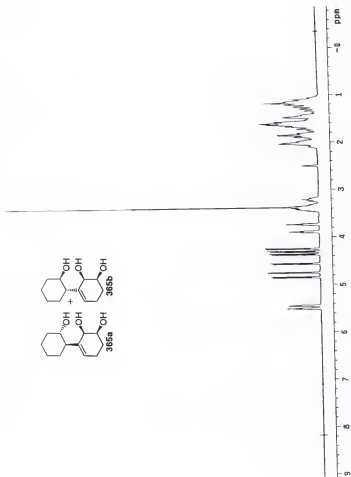


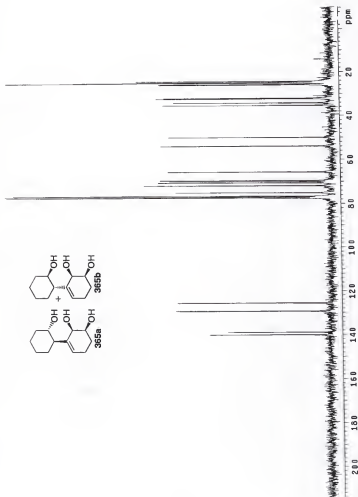


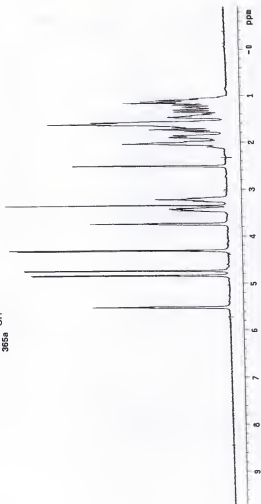


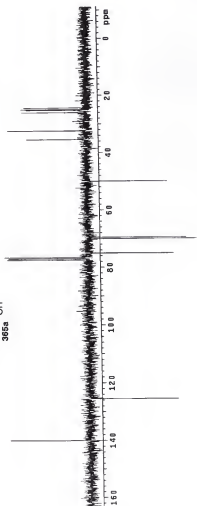


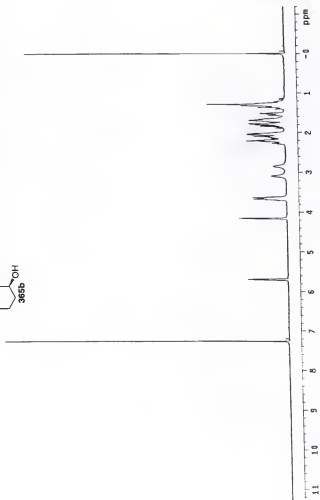


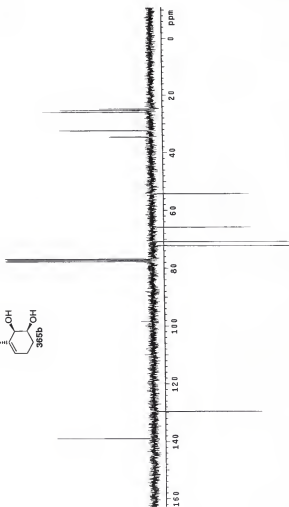


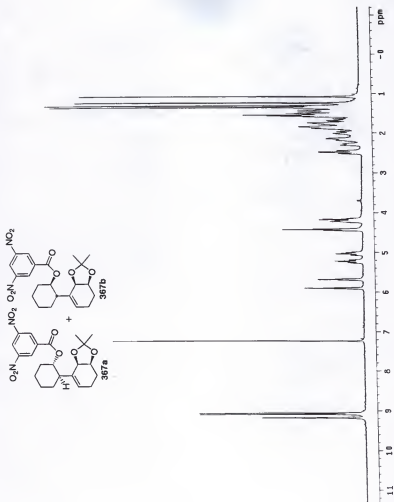


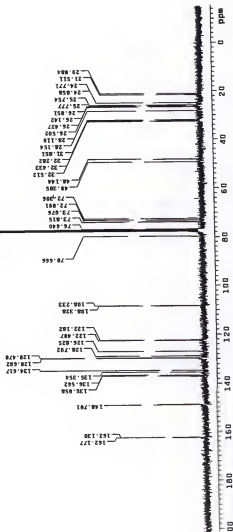
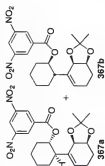


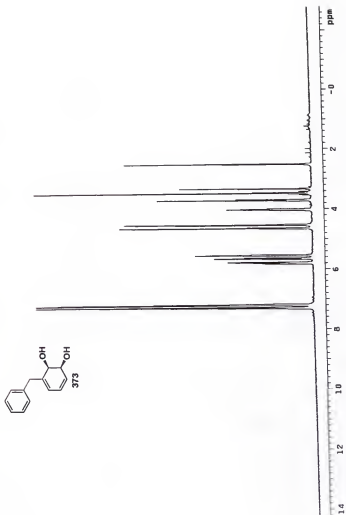


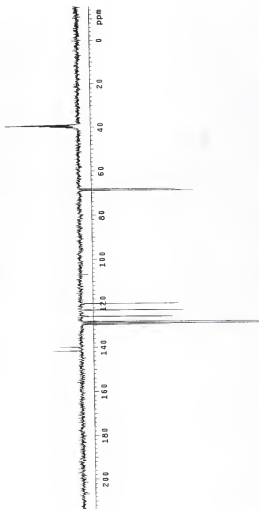


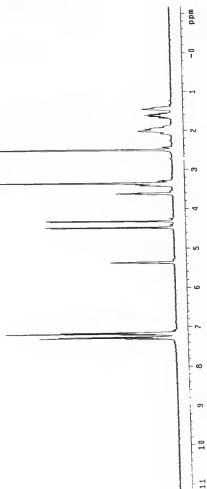


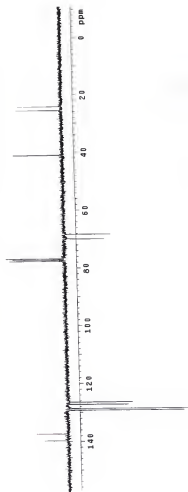


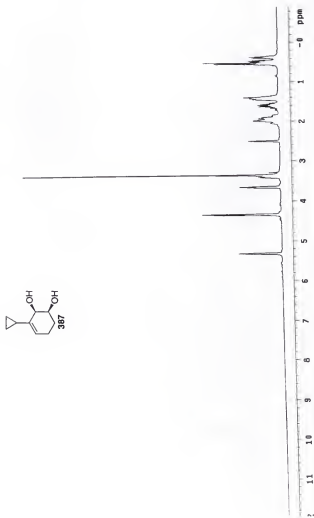


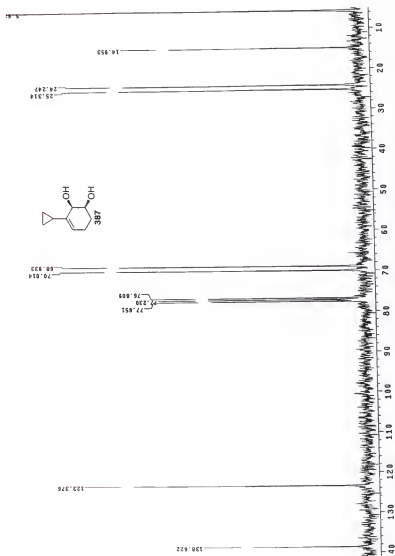




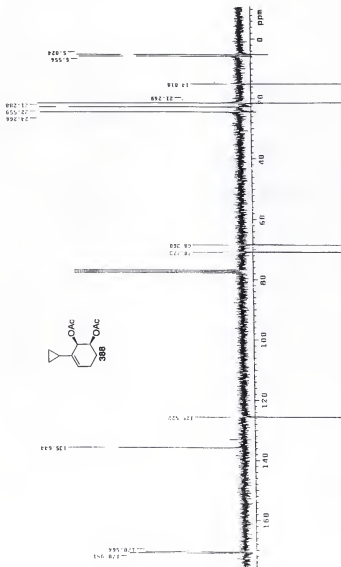


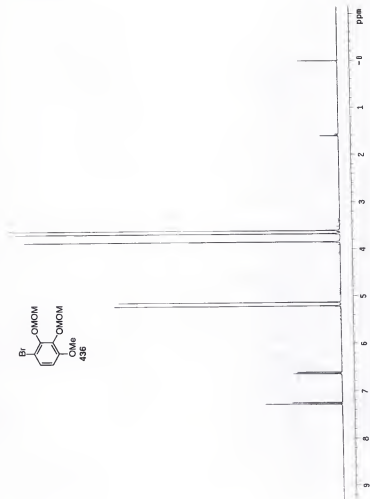


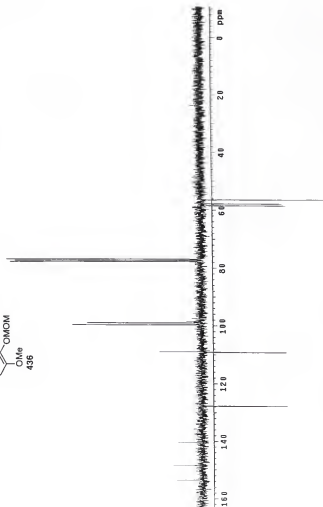


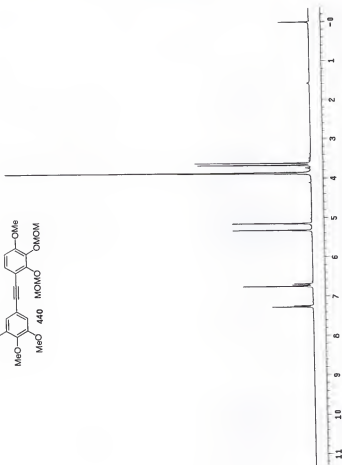
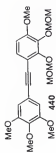


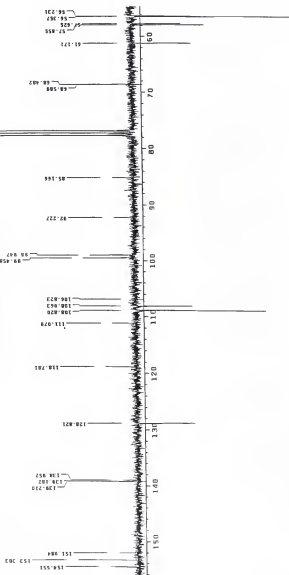
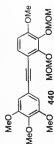


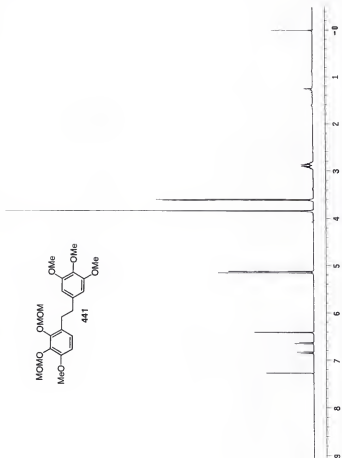


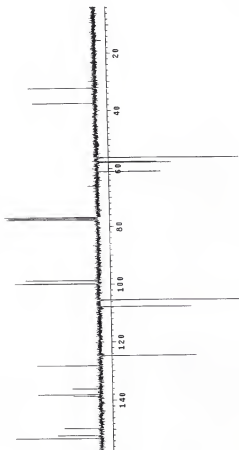
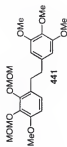


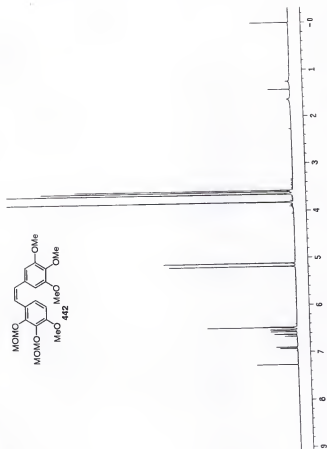


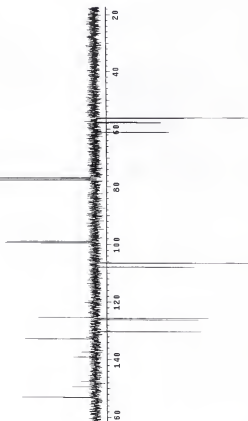
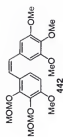


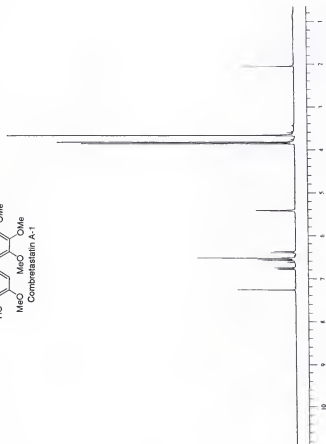
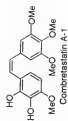


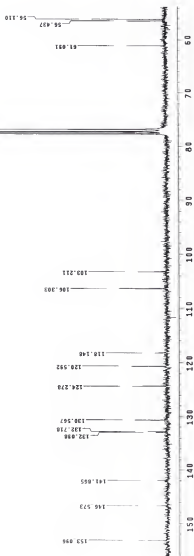
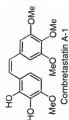


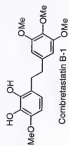




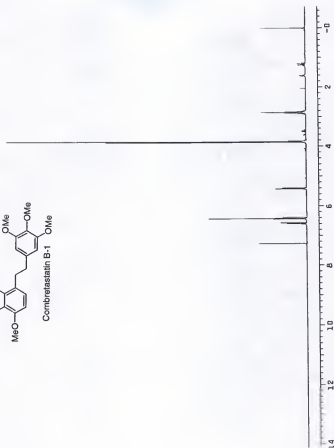


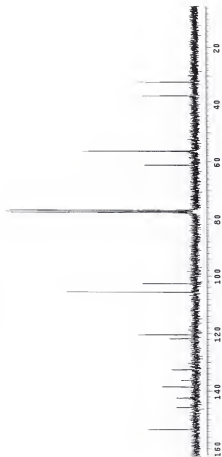
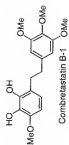


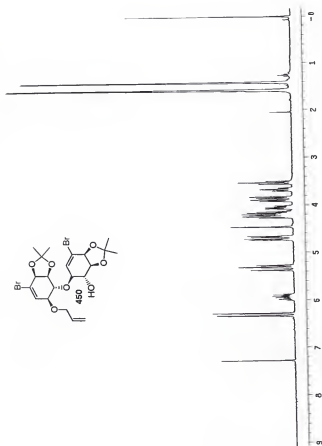


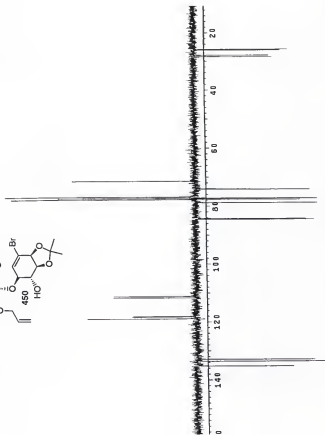
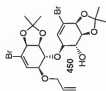


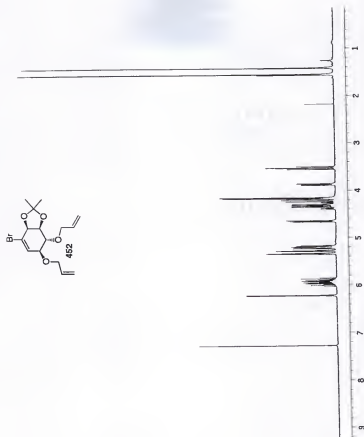
Combretastatin B-1

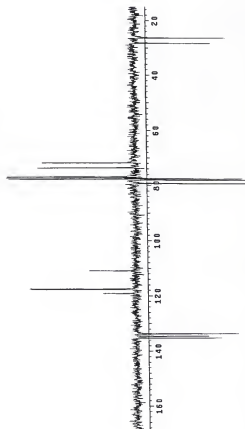


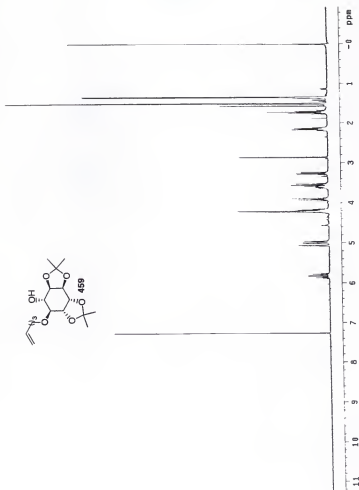




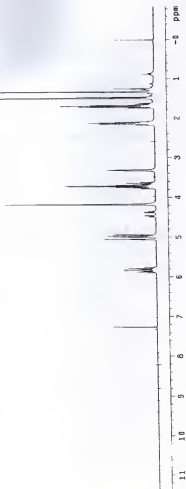


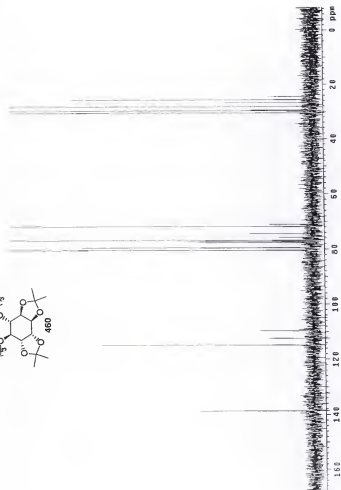


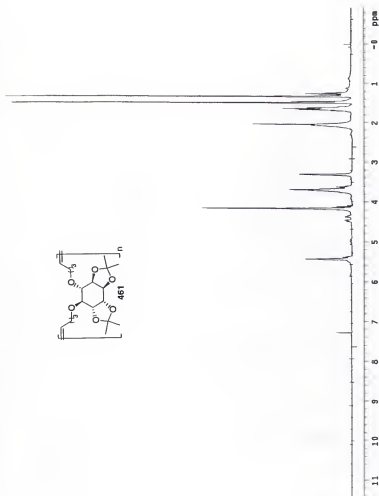












## LIST OF REFERENCES

- (1) Keith, L. H.; Telliard, W. A. *Environ. Sci. Technol.* **1979**, *13*, 416.
- (2) Gibson, D. T.; Subramanian, V. *Microbial Degradation of Organic Compounds*; Marcel Dekker Inc.: New York, 1984.
- (3) Reineke, W. *Annu. Rev. Microbiol.* **1988**, *42*, 263.
- (4) Abraham, W. R.; Stumpf, B.; Arfmann, H. A. *J. Essent. Oil. Res.* **1990**, *2*, 251.
- (5) Boyle, A. W.; Silvin, C. J.; Hasset, J. P.; Nakas, J. P.; Tanenbaum, S. W. *Biodegradation* **1992**, *3*, 285.
- (6) Daly, J. W.; Jerina, D. M.; Witkop, B. *Experientia* **1972**, *28*, 1129.
- (7) Cerniglia, C. E. *Biodegradation* **1992**, *3*, 351.
- (8) Wong, C.-H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 412.
- (9) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994.
- (10) Drauz, K.; Waldmann, H. *Enzyme Catalysis in Organic Synthesis*; VCH publishers: New York, 1995.
- (11) Gijssen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443.
- (12) Fessner, W. D.; Sinerius, G. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 209.
- (13) Nilsson, K. G. I. *Trends in Biotechnol.* **1988**, *6*, 256.
- (14) Okamoto, K.; Goto, T. *Tetrahedron* **1990**, *46*, 5835.
- (15) Roberts, S. M.; Turner, N. J. *Journ. of Biotech.* **1992**, *22*, 227.
- (16) Kadyrov, R.; Selke, R. *Organic Synthesis Highlights IV*, 2000.
- (17) Taschner, M. J.; Black, D. J. *J. Am. Chem. Soc.* **1988**, *110*, 6892.
- (18) Martinez, C. A.; Stewart, J. D. *Current Organic Chemistry*, **2000**, *4*, 263.

- (19) Lintner, C. J.; Liebig, H. J. Z. *Physiol. Chem.* **1913**, *88*, 109.
- (20) Fuganti, C.; Grasselli, P. *Chem. Ind.* **1977**, 983.
- (21) Servi, S. *Synthesis* **1990**, 1.
- (22) Csu, R.; Glanzer, B. I. *Chem. Rev.* **1991**, *91*, 49.
- (23) Seoane, G. *Current Organic Chemistry* **2000**, *4*, 283.
- (24) Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1999**, *32*, 35.
- (25) Sheldrake, G. N. *Chirality and Industry: The commercial Manufacture and Application of Optically Active Compounds*; John Wiley and Sons, LTD: Chichester, UK, **1992**.
- (26) Hudlicky, T.; Reed, J. W. *Advances in asymmetric synthesis*; JAI Press: Greenwich, CT, **1995**; Vol. 1.
- (27) Widdowson, D. A.; Ribbons, D. W.; Thomas, S. D. *Janssen Chimica Acta* **1990**, *8*, 3.
- (28) D'Arrigo, P.; Pedrocchi-Fantoni, G.; Servi, S. In *Stereoselective Biocatalysis*; Marcel Dekker, Inc.: New York, NY., **2000**.
- (29) Gibson, D. T.; Koch, J. R.; Schuld, C. L.; Kallio, R. E. *Biochemistry* **1968**, *7*, 3795.
- (30) Ley, S. V.; Sternfeld, F.; Taylor, S. *Tetrahedron Lett.* **1987**, *28*, 225.
- (31) Ballard, D. G. H.; Courtis, A.; Shirley, I. M.; Taylor, S. C. *J. Chem. Soc., Chem. Commun.* **1983**, 954.
- (32) Ballard, D. G. H.; Courtis, A.; Shirley, I. M.; Taylor, S. C. *Macromolecules* **1988**, *21*, 294.
- (33) Hudlicky, T.; Luna, H.; Barbieri, G.; Kwart, L. D. *J. Am. Chem. Soc.* **1988**, *110*, 4735.
- (34) Johnson, C. R.; Penning, T. D. *J. Am. Chem. Soc.* **1986**, *108*, 5655.
- (35) Johnson, C. R.; Penning, T. D. *J. Am. Chem. Soc.* **1988**, *110*, 4726.
- (36) Davies, I. W.; Senanayake, C. H.; Castonguay, L.; Larsen, R. D.; Verhoeven, T. R.; Reider, P. J. *Tetrahedron Lett.* **1995**, *36*, 7619.
- (37) Davies, I. W.; Reider, P. J. *Chem. Ind.(London)* **1996**, 412.

- (38) Sheldon, R. A. *Chirotechnology: Industrial Synthesis of Optically Active Compounds*; Marcel Dekker, New York, NY., 1993.
- (39) Turner, M. K. *Perspectives in biotransformations*; Wiley-VCH: Weinheim, 1998; Vol. 8.
- (40) Pasteur, L. *Compt. Rend.* **1862**, *55*, 28.
- (41) Pasteur, L. *C. R. Acad. Sci. (Paris)*, **1858**, *46*, 615.
- (42) Sebek, O. K. *Microb. Transform. Bioact. Compd.* **1982**, *1*, 1.
- (43) Brown, A. J. *J. Chem. Soc., Chem. Commun.* **1886**, *49*, 432.
- (44) Buchner, E. *Ber. Dtsch. Chem. Ges.* **1897**, *30*, 1110.
- (45) Prescott, S. C.; Dunn, C. G. *Prescott & Dunn's industrial microbiology*; 4th ed.; AVI Publishing company, INC.: Westport, Conn., 1982.
- (46) Neuberg, C.; Hirsch, J. *Biochem. Z.* **1921**, *115*, 282.
- (47) Beevarova, H.; Hane, O.; Mauk, K. *Folia Microbiol.* **1963**, *8*, 165.
- (48) Behrens, M.; Iwanoff, N. N. *Biochem. Z.* **1921**, *121*, 311.
- (49) Rose, A. H. in *Industrial Microbiology*, Butterworths, Washington, 1961.
- (50) Braun, M.; Wild, H. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 723.
- (51) Kluyver, A. J.; de Leeuw, F. J. *Tijdschr. Verg. Geneesk.* **1924**, *10*, 170.
- (52) Reichstein, T.; Grussner, H. *Helv. Chim. Acta* **1934**, *17*, 311.
- (53) Crueger, W.; Crueger, A.; 2nd ed.; Brock, T. D., Ed.; Sinauer Associates, Inc.: Sunderland, MA, 1989, p 298.
- (54) Peterson, D. H.; Murray, H. C. *J. Am. Chem. Soc.* **1952**, *74*, 1871.
- (55) Peterson, D. H.; Murray, H. C.; Epstein, S. H.; Reinecke, L. M.; Weintraub, A.; Meister, P. D.; Leigh, H. M. *J. Am. Chem. Soc.* **1952**, *74*, 5933.
- (56) Charney, W.; Herzog, H. L. *Microbial transformations of Steroids*; Academic Press: New York, 1967.
- (57) Crueger, W.; Crueger, A.; 2nd ed.; Brock, T. D., Ed.; Sinauer Associates, Inc.: Sunderland, MA, 1989, p 294.
- (58) Sarett, L. H. *J. Biol. Chem.* **1946**, *162*, 601.

- (59) Sebek, O. K.; Perlman, D. In *Microb. Technol.*; 2nd ed. 1979; Vol. 1, p 483-488.
- (60) Liese, A.; Seelbach, K.; Wandrey, C.; VCH-Wiley: New York, 2000, p 10.
- (61) Nunn, J. F. In *Oxygen: in applied respiratory physiology*, 2nd ed. Boston, Betterworths, 1977, p 375.
- (62) Dix, T. A.; Benkovic, S. S. *Acc. Chem. Res.* **1988**, *21*, 101.
- (63) Dawson, J. H.; Sono, M. *Chem. Rev.* **1987**, *87*, 1255.
- (64) Sato, R.; Omura, T. *cytochrome P-450*; Academic Press: New York., 1978.
- (65) *cytochrome P-450*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986.
- (66) Omura, T.; Ishimura, Y.; Fujii-Kuriyama, Y. *Cytochrome P-450*; Second ed.; VCH: Tokyo, 1992.
- (67) Garfinkel, D. *Arch. Biochem. Biophys.* **1957**, *71*, 111.
- (68) Klingenberg, M. *Arch. Biochem. Biophys.* **1958**, *75*, 376.
- (69) Estabrook, R. W.; Cooper, D. Y.; Rosenthal, O. *Biochem. Z.* **1963**, *338*, 741.
- (70) Tan, L.; Muto, N. *Eur. J. Biochem.* **1986**, *156*, 243.
- (71) Shikita, M.; Hall, P. F. *J. Biol. Chem.* **1973**, *248*, 5598.
- (72) Katagiri, M.; Ganguli, B. M.; Gunsalus, I. C. *J. Biol. Chem.* **1968**, *243*, 3543.
- (73) Guengerich, F. P.; Kim, D.; Iwasaka, M. *Chem. Res. Toxicol.* **1991**, *4*, 168.
- (74) Estabrook, R. W.; Hildebrandt, A. G.; Baron, J.; Netter, K. J.; Leibman, K. *Biochem. Biophys. Res. Commun.* **1971**, *42*, 132.
- (75) Poulos, T. L.; Finzel, B. C.; Howard, A. J. *J. Mol. Biol.* **1987**, *195*, 687.
- (76) Poulos, T. L.; Finzel, B. C.; Howard, A. J. *Biochemistry* **1986**, *25*, 53140.
- (77) Sligar, S. G. *Biochemistry* **1976**, *15*, 5399.
- (78) Kassner, R. J. *J. Am. Chem. Soc.* **1973**, 2674.
- (79) Mueller, E. J.; Loida, P. J.; Sligar, S. G.; 2nd ed.; Plenum: New York, 1995, p 83.
- (80) Ryerson, C. C.; Ballou, D. P.; Walsh, C. *Biochemistry* **1982**, *21*, 2644.
- (81) Visser, C. M. *Eur. J. Biochem.* **1983**, *135*, 543.

- (82) Ghisla, S.; Massey, V. *Eur. J. Biochem.* **1989**, *181*, 1.
- (83) Mansuy, D.; Battioni, P.; Wiley: New York, 1989, p 195.
- (84) Crabtree, R. H. *Chem. Rev.* **1985**, *85*, 245.
- (85) Shilov, A. E. *Activation of Saturated Hydrocarbons by Transition Metal Complexes*; D. Reidel Publishing: Dordrecht, 1984.
- (86) Sheldon, R. A.; Kochi, J. K. *Metal-Catalyzed oxidation of organic Compounds*; Academic Press: New York, 1981.
- (87) Breslow, R. *Acc. Chem. Res.* **1980**, *13*, 170.
- (88) Fossey, J.; Lefort, D.; Massoudi, M.; Nedelec, J.-Y.; Sorba, J. *Can. J. Chem.* **1985**, *63*, 678.
- (89) Barton, D. H. R.; Kalley, F.; Ozbalik, N.; Young, E.; Balavoine, G. *J. Am. Chem. Soc.* **1989**, *111*, 7144.
- (90) Johnson, R. A.; Trahanovsky, W. S., Ed.; Academic Press: New York, 1978, p 131.
- (91) Dalton, H. *Adv. Appl. Microbiol.* **1980**, *26*, 71.
- (92) Kieslich, K. *Bull. Soc. Chim. Fr.* **1980**, *11*, 9.
- (93) Sariaslani, F. S. *Crit. Rev. Biotechnol.* **1989**, *9*, 171.
- (94) Ruce, N. C.; Franch, C. E.; Hailes, A. M.; Long, M. T.; Rathbone, D. A. *Trends Biotechnol.* **1995**, *13*, 200.
- (95) Ribbons, D. T.; Michalover, J. L. *FEBS Letters* **1970**, *11*, 41.
- (96) Colby, J.; Stirling, D.; Dalton, H. *Biochemical journal* **1977**, *165*, 395-402.
- (97) Colby, J.; Dalton, H. *Biochemical Journal* **1979**, *177*, 903-908.
- (98) Tonge, G. M.; Harrison, D. E. F.; Higgins, I. J. *Biochemical Journal* **1977**, *161*, 333.
- (99) Burrows, K., J.; ; Cornish, A.; Scott, D.; Hoggins, I. J. *J. Gen. Microbiol.* **1984**, *130*, 3327.
- (100) Rosazza, J. P. N.; Steffens, J. J.; Sariaslani, S.; Goswami, A.; Beale, J. M.; Reeg, S.; Chapman, R. *Appl. Environ. Microbiol.* **1987**, *53*, 2482.
- (101) Liu, W. G.; Goswami, A.; Steffek, R. P.; Chapman, R. L.; Sariaslani, F. S.; Steffens, J. J.; Rosazza, J. P. N. *J. Org. Chem.* **1988**, *53*, 5700.

- (102) Weiler, E. W.; Droste, M.; Eberle, J.; Halfmann, H. J.; Weber, A. *Appl. Microbiol. Biotechnol.* **1987**, *27*, 252.
- (103) Perlman, D.; Titius, E.; Fried, J. *J. Am. Chem. Soc.* **1952**, *74*, 2126.
- (104) Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M.; Academic Press: London, 1989, p 175.
- (105) Fried, J.; Thoma, R. W.; Gerke, J. R.; Herz, J. E.; Donin, M. N.; Perlman, D. *J. Am. Chem. Soc.* **1952**, *74*, 3692.
- (106) Sawada, S.; Kulprecha, S.; Nilubol, N.; Yoshida, T.; Knoshita, S.; Taguchi, H. *Appl. Environ. Microbiol.* **1982**, *44*, 1249.
- (107) Olah, G. A.; Ernst, T. D. *J. Org. Chem.* **1989**, *54*, 1204.
- (108) Komiyam, A. A. *J. Chem. Soc., Perkin Trans. I* **1989**, 2031.
- (109) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.
- (110) Adachi, K.; Takeda, Y.; Senoh, S.; Kita, H. *Biochemica et Biophysica Acta* **1964**, *93*, 483.
- (111) Michalover, J. L.; Ribbons, D. W. *Biochemical and biophysical Research Communications* **1973**, *55*, 888.
- (112) Groseclo, E. E.; Ribbons, D. W. *Biochemical and Biophysical Research Communications* **1973**, *55*, 897.
- (113) Wang, L. H.; Harnzah, R. Y.; Yu, Y.; Tu, S. C. *Biochemistry* **1987**, *26*, 1099.
- (114) Powlowski, J. B.; Dagley, S.; Massey, V.; Ballou, D. P. *J. Biol. Chem.* **1987**, *262*, 69.
- (115) Wiseman, A.; King, D. J. *Topic Enzymol. Ferment. Biotechnol.* **1982**, *6*, 151.
- (116) Daly, J. W.; Jerina, D. M.; Witkop, B. *Experientia* **1973**, 1129.
- (117) Boyd, D. R.; Campbell, R. M.; Craig, H. C.; Watson, C. G.; Daly, J. W.; Jerina, D. M. *J. Chem. Soc. Perkin Trans. I* **1976**, 2438.
- (118) Klibanov, A. M.; Berman, Z.; Alberti, B. N. *J. Am. Chem. Soc.* **1981**, *103*, 6263.
- (119) Agarwal, M.; Shukla, O. P. *Journal of Microbial Biotechnology* **1988**, *3*, 18.
- (120) Factor, S. A.; Schneider, A. S. *Experimental Neurology* **1995**, *131*, 64.
- (121) Pasutto, F. M.; Singh, N. N. J.; amali, F.; Coutts, R. T.; Abuzar, S. *J. Pharm. Sci.* **1987**, *76*, 177.

- (122) Wigne, B.; Archelas, A.; Furstoss, R. *Tetrahedron* **1991**, *47*, 1447.
- (123) Yoshioka, H.; Nagasawa, T.; Hasegawa, R.; Yamada, H. *Biotechnol. Lett.* **1990**, *679*.
- (124) Theriault, R. J.; Longfield, T. H. *Appl. Microbiol. Biotechnol.* **1973**, *25*, 606.
- (125) Glockler, R.; Roduit, J.-P. *Chimia* **1996**, *50*, 413.
- (126) Watson, G. K.; Houghton, C.; Cain, R. B. *Biochem. J.* **1974**, *140*, 265.
- (127) Schurig, V.; Betschinger, F. *Chem. Rev.* **1992**, *92*, 873.
- (128) Johnson, R. A.; Sharpless, K. B.; Ojima, I., Ed.; Verlag Chemie: New York, 1993, p 103-158.
- (129) Jacobsen, E. N.; Zhang, W.; Muci, A. R.; Ecker, J. R.; Deng, L. *J. Am. Chem. Soc.* **1991**, *113*, 7063.
- (130) Konish, K.; Oda, K.; Nishida, K.; Aida, T.; Inoue, S. *J. Am. Chem. Soc.* **1992**, *114*, 1313.
- (131) de Bont, J. A. M. *Tetrahedron: Asymetry* **1993**, *4*, 1331.
- (132) Leak, D. J.; J., A. P.; Seyed-Mahmoudian, M. *Trends, Biotechnol.* **1992**, *10*, 256.
- (133) Habets-Clutzen, A. Q. H.; Carlier, S. J. N.; de Bont, J. A. M.; Wistuba, D.; Schurig, V.; Hartmans, S.; Trumper, J. *Enzyme Microb. Technol.* **1985**, *7*, 17.
- (134) de Smet, M.-J.; Witholt, B.; Wynberg, H. *enzyme Microb. Technol.* **1983**, *5*, 352.
- (135) Jurtshuk, P.; Cardini, G. E. *Crit. Rev. Microbiol.* **1972**, *1*, 254.
- (136) Hou, C. T.; Patel, R.; Laskin, A. I.; Barnabe, N.; Barist, I. *Appl. Environ. Microbiol.* **1983**, *46*, 171.
- (137) Ohta, H.; Tetsukawa, H. *Agric. Biol. Chem.* **1979**, *43*, 2099.
- (138) Johnstone, S. L.; Phillips, G. T.; Robertson, B. W.; Watts, P. D.; Bertola, M. A.; Koger, H. S.; Marx, A. F. *Stereoselective Synthesis of (S)-B-Blockers via Microbially Produced epoxide intermediates. In Biocatalysis in Organic media*; Elsevier: Amsterdam, 1987.
- (139) Fu, H.; Newcomb, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 5878.
- (140) Howe, R.; Shanks, R. G. *Nature* **1966**, *210*, 1336.
- (141) Weijers, C. A. G. M.; van Ginkel, C. G.; de Bont, J. *Enzyme Microb. Technol.* **1988**, *10*, 214.

- (142) Furuhashi, K. *Biological Methods to Optically active epoxides. In Chirality in Industry*; Wiley: New York, 1992.
- (143) Takahashi, O.; Umezawa, J.; Furuhashi, K.; Takagi, M. *Tetrahedron Lett.* **1989**, 30, 1583.
- (144) Furuhashi, K. *Chem. Econ. Eng. Rev.* **1986**, 18, 7.
- (145) White, R. F.; Birnbaum, J.; Meyer, R. T.; ten Broeke, J.; Chemerda, J. M.; Demain, A. L. *Appl. Microbiol. Biotechnol.* **1971**, 22, 55.
- (146) Baeyer, A.; Villiger, V. *Berichte* **1899**, 32, 3625.
- (147) Criegee, R. *Liebigs Ann. Chem.* **1948**, 560, 127.
- (148) Lee, J. B.; Uff, B. C. *Quart. Rev.* **1967**, 21, 429.
- (149) Leffler, J. E. *Chem. Rev.* **1949**, 45, 385.
- (150) Hassall, C. H. *Org. React.* **1957**, 9, 73.
- (151) House, H. O. In *Modern Synthetic Reactions*; Benjamin: New York, 1972, p 327.
- (152) Plesnicar, B. *Oxidation in Organic Chemistry, Part C* New York, 1978.
- (153) Schwab, J. M.; B., L. W.; Thomas, L. P. *J. Am. Chem. Soc.* **1983**, 105, 4800.
- (154) Willetts, A. *Trends in Biotechnology* **1997**, 15, 55.
- (155) Nealson, K. H.; Hastings, J. W. *Microbiol. Rev.* **1979**, 43, 496.
- (156) Chen, Y.-C. J.; Peoples, O. P.; Walsh, C. T. *J. Bacteriol.* **1988**, 170, 781.
- (157) Donoghue, N. A.; Norris, D. B.; Trugill, P. W. *Eur. J. Biochem.* **1976**, 63, 175.
- (158) Fried, J.; Toma, R. W.; Klingsberg, A. *J. Am. Chem. Soc.* **1953**, 75, 5764.
- (159) Gunsalus, I. C.; Peterson, T. C.; Sligar, S. G. *Ann. Rev. Biochem.* **1975**, 44, 377.
- (160) Wash, C. T.; Chen, Y.-C. *J. Angew. Chem., Int. Ed. Engl.* **1988**, 27, 333.
- (161) Roberts, S. M.; Wan, P. W. H. *J. Mol. Catal.* **1998**, B4, 111.
- (162) Alphand, V.; Furstoss, R.; Drauz, K.; Waldmann, H., Eds.; Wiley-VCH: New York, 1995, p 745-772.
- (163) Carnell, A. J.; Roberts, S. M.; Sik, V.; Willetts, A. *J. Chem. Soc. Perkin Trans. I* **1991**, 2385.

- (164) Konigsberger, K.; Griengl, H. *Bioorg. Med. Chem.* **1994**, *2*, 595.
- (165) Petit, F.; Furstoss, R. *Synthesis* **1995**, 1517.
- (166) Taschner, M. J.; J.; B. D.; Chen, Q.-Z. *Tetrahedron: Asymmetry* **1993**, *4*, 1387.
- (167) Kelly, D. R.; Knowles, C. J.; Mahdi, J. G.; Taylor, I. N.; Wright, M. A. *J. Chem. Soc., Chem. Commun.* **1995**, 729.
- (168) Ottolina, G.; Carrea, G.; Colonna, S.; Ruckemann, A. *Tetrahedron: Asymmetry* **1996**, *7*, 1123.
- (169) Ouazzani-Chahdi, J.; Buisson, D.; Azerad, R. *Tetrahedron Lett.* **1987**, *28*, 1109.
- (170) Alphand, V.; Archelas, A.; Furstoss, R. *Biocatalysis* **1990**, *3*, 73.
- (171) Levitt, M. S.; Newton, R. F.; Roberts, S. M.; Willetts, A. J. *J. Chem. Soc., chem. Commun.* **1990**, 619.
- (172) Stewart, J. D.; Reed, K. W.; Kayser, M. M. *J. Chem. Soc., Perkin Trans. I* **1996**, 755.
- (173) Stewart, J. D.; Reed, K. W.; Martinez, C. A.; Zhu, J.; Chen, G.; Kayser, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 3541.
- (174) Kayser, M. M.; Chen, G.; Stewart, J. D. *J. Org. Chem.* **1998**, *63*, 7103.
- (175) Kayser, M.; Chen, G.; Stewart, J. D. *Synlett.* **1999**, 153.
- (176) Redmond, D. E. J. N. *Engl. J. Med.* **1970**, *282*, 18.
- (177) Pott, P. *Natl. Cancer Inst. Monogr.* **1963**, *10*, 7.
- (178) Kennaway, E. L.; Heiger, I. *Br. Med. J.* **1930**, *1*, 1044.
- (179) Cook, J. W.; Hewett, L.; Heiger, I. *J. Chem. Soc. Perkin Trans. I* **1933**, 395.
- (180) Freudenthal, R. I.; Jones, P. W. *Carcinogenesis: A comprehensive Survey*; Raven Press: New York, 1978; Vol. 1.
- (181) Higginson, J.; Muir, C. S. *Cancer Medicine*; Lea and Febiger: Philadelphia, 1973.
- (182) Dagley, S. *In the Bacteria*; Sokatch, J. R., Ed.; Academic: London, 1986; Vol. 10, p 527-55.
- (183) Jeffrey, A. M.; Yeh, H. J. C.; Jerina, D. M.; Patel, T. R.; Davey, J. F.; Gibson, D. T. *Biochemistry* **1975**, *14*, 575.

- (184) Gibson, D. T.; Subramanian, V. *Microbial degradation of Organic Molecules*; Marcel Dekker: New York, 1984.
- (185) Lipscomb, J. D.; Orville, A. M. *Metal Ions Biol. Syst.* **1992**, 28, 243.
- (186) Que, L., Jr.; Loehr, T. M., Ed.; VCH: New York, 1989, p 467-524.
- (187) Que, L., Jr.; Vidom, J.; Crawford, R. L. *J. Biol. Chem.* **1981**, 256, 10941.
- (188) Boldt, Y. R.; Sadowsky, M. J.; Ellis, L. B. M.; Que, L., Jr.; Wackett, L. P. *J. Bacteriol.* **1995**, 177, 1225.
- (189) Whiting, A. K.; Boldt, Y. R.; Hendrich, M. P.; Wackett, L. P.; Que, L., Jr. *Biochemistry* **1996**, 35, 160.
- (190) Gibson, D. T.; Chapman, P. J. *Critical Reviews in Microbiology* **1971**, 199.
- (191) Gibson, D. T.; Zylstra, G. J.; Chauhan, S. *Pseudomonas: biotransformations, Pathogenesis and Evolving Biochemistry*; American Society for Microbiology, 1990.
- (192) Resnick, S. M.; Lee, K.; Gibson, D. T. *J. Ind. Microbiol.* **1996**, 17, 438.
- (193) Butler, C. S.; Mason, J. R. *Adv. Microb. Physiol.* **1997**, 38, 48.
- (194) Gibson, D. T.; Koch, J. R.; Kallio, R. E. *Biochemistry* **1968**, 7, 2653.
- (195) Rossiter, J. T.; Williams, S. R.; Cass, A. E. G.; Ribbons, D. W. *Tetrahedron Lett.* **1987**, 28, 5173.
- (196) Reineke, W.; Knackmuss, H. J. *Biochim. Biophys. Acta* **1987**, 542, 412.
- (197) Whited, G. M.; McCombie, W. R.; Kwart, L. D.; Gibson, D. T. *J. Bacteriol.* **1986**, 166, 1028.
- (198) Khan, A. A.; Wang, R. F.; Cao, W. W.; Franklin, W.; Cerniglia, C. E. *Int. J. Sys. Bacteriol.* **1996**, 46, 466.
- (199) Reiner, A. M.; Hegeman, G. D. *Biochemistry* **1971**, 10, 2530.
- (200) Zylstra, G. J.; Gibson, D. T. *J. Biol. Chem.* **1989**, 264, 14940.
- (201) Allen, C. C. R.; Boyd, d. R.; Dalton, H.; Sharma, N. D.; Haughey, S. A.; McMordie, R. A. S.; McMurray, B. T.; Sheldrake, G. N.; Sproule, K. *Chem. Commun.* **1995**, 119.
- (202) Gibson, D. T.; Cardini, G. E.; Maseles, F. C.; Kallio, R. E. *Biochemistry* **1970**, 9, 1631-5.

- (203) Gibson, D. T.; Hensley, M.; Yoshioka, H.; Mabry, T. J. *Biochemistry* **1970**, *9*, 1626.
- (204) Kobal, V. M.; Gibson, D. T.; Davis, R. E.; Garza, A. J. *Am. Chem. Soc.* **1973**, *95*, 4420.
- (205) Yeh, W. K.; Gibson, D. T.; Liu, T. N. *Biochem. Biophys. Res. Commun.* **1977**, *78*, 401.
- (206) Subramanian, V.; Liu, T. N.; Yeh, W. K.; Narro, M.; Gibson, D. T. *J. Biol. Chem.* **1981**, *256*, 2723.
- (207) Subramanian, V.; Liu, T. N.; Yeh, W. K.; Serdar, C. M.; Wackett, L. P.; Gibson, D. T. *J. Biol. Chem.* **1985**, *260*, 2355.
- (208) Subramanian, V.; Liu, T. N.; Yeh, W. K.; Gibson, D. T. *Biochem. Biophys. Res. Commun.* **1979**, *91*, 1131.
- (209) Rogers, J. E.; Gibson, D. T. *J. Bacteriol.* **1977**, *130*, 1117.
- (210) Finette, B. A.; Subramanian, V.; Gibson, D. T. *J. Bacteriol.* **1984**, *160*, 1003.
- (211) Finette, B. A.; Gibson, D. T. *Biocatalysis* **1988**, *2*, 29.
- (212) Boyd, D. R.; Sharma, N. D.; Hand, M. V.; Groocock, M. R.; Kerley, N. A.; Dalton, H.; Chima, J.; Sheldrake, G. N. *Chem. Commun.* **1993**, 974.
- (213) Boyd, D. R.; Sharma, N. D.; Barr, S. A.; Dalton, H.; Chima, J.; Whited, G.; Seemayer, R. J. *Am. Chem. Soc.* **1994**, *116*, 1147.
- (214) Boyd, D. R.; McMordie, R. A. S.; Porter, H. P.; Dalton, H.; Jenkins, R. O.; Howarth, O. W. *J. Chem. Soc., Chem. Commun.* **1987**, 1722.
- (215) Jerina, D. M.; Selander, H.; Yagi, H.; Wells, M. C.; Davey, J.; Mahadevan, V.; Gibson, D. T. *J. Am. Chem. Soc.* **1976**, *98*, 5988.
- (216) Koreeda, M.; Akhtar, M. N.; Boyd, D. R.; Neill, J. D.; Gibson, D. T.; Jerina, D. M. *J. Org. Chem.* **1978**, *43*, 1023.
- (217) Boyd, D. R.; Sharma, N. D.; Dorrity, M. R. J.; Hand, M. V.; McMordie, R. A. S.; Malone, J. F.; Dalton, H.; Chima, J.; Sheldrake, G. N. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1065.
- (218) Boyd, D. R.; Sharma, N. D.; Boyle, R.; McMurray, B. T.; Evans, T. A.; Malone, J. F.; Chima, J.; Dalton, H.; Sheldrake, G. N. *J. Chem. Soc., Chem. Commun.* **1993**, 49.
- (219) Boyd, D. R.; Sharma, N. D.; Brannigan, I. N.; Clarke, D. A.; Dalton, H.; Haughey, S. A.; Malone, J. F. *Chem. Commun.* **1996**, 2361.

- (220) Boyd, D. R.; Sharma, N. D.; Boyle, R.; Malone, J. F.; Chima, J.; Dalton, H. *Tetrahedron: Asymmetry* **1993**, *4*, 1307.
- (221) Resnick, S. M.; Torok, D. S.; Gibson, D. T. *J. Org. Chem.* **1995**, *60*, 3546.
- (222) Boyd, D. R.; Dorrity, M. R. J.; Hand, M. V.; Malone, J. F.; Sharma, N. D.; Dalton, H.; Gray, D. J.; Sheldrake, G. N. *J. Am. Chem. Soc.* **1991**, *113*, 666.
- (223) Jenkins, N.; Ribbons, D. W.; Widdowson, D. A.; Slavin, A. M. Z.; Williams, D. J. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2647.
- (224) Boyd, R.; Sharma, N. D.; Agarwal, R.; Resnick, S. M.; Schoken, M. J.; Gibson, D. T.; Sayer, J. M.; Yagi, H.; Jerina, D. M. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1715.
- (225) Kumar, A.; Ernst, R. R.; Wuthrich, K. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1.
- (226) Boyd, D. R.; Sheldrake, G. N. *Nat. Prod. Rep.*, **1998**, *15*, 309.
- (227) Bowers, N. Ph.D. Thesis, The Queen's University of Belfast, 1997.
- (228) Ley, S. V.; Sternfeld, F. *Tetrahedron* **1989**, *45*, 3463.
- (229) Ley, S. V.; Parra, M.; Redgrave, A. J.; Sternfeld, F. *Tetrahedron* **1990**, *46*, 4995.
- (230) Hudlicky, T.; Luna, H.; Price, J. D.; Rulin, F. *Tetrahedron Lett.* **1989**, *30*, 4053.
- (231) Hudlicky, T.; Price, J. D. *Synlett*, **1990**, 159.
- (232) Hudlicky, T.; Luna, H.; Price, J. D.; Rulin, F. *J. Org. Chem.* **1990**, *55*, 4683.
- (233) Hudlicky, T.; Rulin, F.; Tsunoda, T.; Luna, H.; Andersen, C.; Price, J. D. *Isr. J. Chem.* **1991**, *31*, 229.
- (234) Hudlicky, T.; Olivo, H. F. *Tetrahedron Lett.* **1991**, *32*, 6077.
- (235) Johnson, C. R.; Ple, P. A.; Su, L.; Heeg, M. J.; Adams, J. P. *Synlett* **1992**, 388.
- (236) Mandel, M.; Hudlicky, T.; Kwart, L. D.; Whited, G. M. *J. Org. Chem.* **1993**, *58*, 2331.
- (237) Hudlicky, T.; Price, J. D.; Rulin, F.; Tsunoda, T. *J. Am. Chem. Soc.* **1990**, *112*, 9439.
- (238) Hudlicky, T.; Pitzer, K. K.; Stabile, M. R.; Thorpe, A. J.; Whited, G. M. *J. Org. Chem.* **1996**, *61*, 4151.

- (239) Banwell, M.; Blakey, S.; Harfoot, G.; Longmore, R. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3141.
- (240) Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. *J. Chem. Rev.* **1996**, 96, 1195.
- (241) Rouden, J.; Hudlicky, T. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1095.
- (242) Hudlicky, T.; Rouden, J.; Luna, H.; Allen, S. *J. Am. Chem. Soc.* **1994**, 116, 5099.
- (243) Hudlicky, T.; Natchus, M. *J. Org. Chem.* **1992**, 57, 4740.
- (244) Banwell, M. G.; McRae, K. *J. Org. Lett.* **2000**, 2, 3583.
- (245) Tian, X.; Hudlicky, T.; Konigsberger, K. *J. Am. Chem. Soc.* **1995**, 117, 3643.
- (246) Hudlicky, T.; Tian, X.; Konigsberger, K.; Maurya, R.; Rouden, J.; Fan, B. *J. Am. Chem. Soc.* **1996**, 118, 10752.
- (247) Tian, X.; Maurya, R.; Konigsberger, K.; Hudlicky, T. *Synlett* **1995**, 1125.
- (248) Hudlicky, T.; Olivo, H. F. *J. Am. Chem. Soc.* **1992**, 114, 9694.
- (249) Hudlicky, T.; Olivo, H. F.; McKibben, B. *J. Am. Chem. Soc.* **1994**, 116, 5108.
- (250) Gonzalez, D.; Shapiro, V.; Seoane, G.; Hudlicky, T.; Abboud, K. *J. Org. Chem.* **1997**, 62, 1194.
- (251) Hudlicky, T.; Abboud, K. A.; Entwistle, D. A.; Fan, R.; Maurya, R.; Thorpe, A. J.; Bolonick, J.; Myers, B. *Synthesis* **1996**, 897.
- (252) Banwell, M. G.; McRae, K. *J. Org. Chem.* **2001**, 66, 6768.
- (253) Johns, B. A.; Pan, Y. T.; Elbein, A. D.; Johnson, C. R. *J. Am. Chem. Soc.* **1997**, 119, 4856.
- (254) Banwell, M.; McLeod, M. *Chem. Commun.* **1998**, 1851.
- (255) Banwell, M. G.; Darnos, P.; McLeod, M. D.; Hockless, D. C. R. *Synlett* **1998**, 897.
- (256) Butora, G.; Gum, A. G.; Hudlicky, T.; Abboud, K. A. *Synthesis* **1998**, 275.
- (257) Hudlicky, T.; Seoane, G.; Pettus, T. *J. Org. Chem.* **1989**, 54, 4239.
- (258) Hudlicky, T.; Boros, C. H. *Tetrahedron Lett.* **1993**, 34, 2557.
- (259) Frey, D. A.; Duan, C.; Hudlicky, T. *Org. Lett.* **1999**, 1, 2085.

- (260) Butora, G.; Hudlicky, T.; Fearnley, S. P.; Gum, A. G.; Stabile, M. R.; Abboud, K. *Tetrahedron Lett.* **1996**, 37, 8155.
- (261) Butora, G.; Hudlicky, T.; Fearnley, S. P.; Stabile, M. R.; Gum, A. G.; Gonzales, D. *Synthesis* **1998**, 665.
- (262) Tran, C. H.; Crout, D. H. G.; Errington, W.; Whited, G. M. *Tetrahedron: Asymmetry* **1996**, 7, 691.
- (263) Stabile, M. R.; Hudlicky, T.; Meisels, M. L.; Butora, G.; Gum, A. G.; Fearnley, S. P.; Thorpe, A. J.; Ellis, M. R. *Chirality* **1995**, 7, 556.
- (264) Johnson, C. R.; Ple, P. A.; Adams, J. P. *J. Chem. Soc., Chem. Commun.* **1991**, 1006.
- (265) Johnson, C. R.; Adams, J. P.; Collins, M. A. *J. Chem. Soc., Perkin Trans. I* **1993**, 1.
- (266) Mandel, M.; Hudlicky, T. *J. Chem. Soc., Perkin Trans. I* **1993**, 13, 1537.
- (267) Hudlicky, T.; Restrepo-Sanchez, N.; Kary, P. D.; Jaramillo-Gomez, L. M. *Carbohydr. Res.* **2000**, 324, 200.
- (268) Ensley, B. D.; Ratzkin, B. J.; Osslund, T. D.; Simon, M. J.; Wackett, L. P.; T., G. D. *Science* **1983**, 222, 167.
- (269) Cavalca, L.; Gilardi, F. *Atti Soc. Lomb. Sci. Med. Biol.* **1955**, 154.
- (270) Winterhoff, H.; Gumbinger, H. G.; Sourgens, H. *Planta Med.* **1988**, 54, 101.
- (271) Siddiqui, B. S.; Adil, Q.; Siddiqui, S. *Pak. J. Pharm. Sci.* **1993**, 6, 53.
- (272) Ladd, D. L.; Weinstock, J. J. *J. Org. Chem.* **1981**, 46, 203.
- (273) Ladd, D. L.; Gaitanopoulos, D.; Weinstock, J. J. *Synth. Commun.* **1985**, 15, 61.
- (274) Murakami, M.; Chen, J. C. *Bull. Chem. Soc. Jpn.* **1963**, 36, 263.
- (275) Perkin and Robinson *J. Chem. Soc. Perkin Trans. I* **1914**, 105, 2383.
- (276) Ide, B. a.; Blatt, A. H., Ed.; John Wiley & Sons, Inc.: London, 1943; Vol. II, p 44.
- (277) Bigelow, A. *organic Syntheses*; John Wiley and Sons, Inc.: New York, N. Y., 1941; Vol. 2.
- (278) Dawson; Wasserman; Keil *J. Am. Chem. Soc.* **1946**, 68, 534.
- (279) Klix, R. C.; Chain, M. H.; Bhatia, A. V. *Tetrahedron Lett.* **1995**, 36, 6413.

- (280) Balz and Schiemann *Ber. Dtsch. Chem. Ges.* **1927**, 60, 1186.
- (281) Corse, J.; Ingraham, L. L. *J. Org. Chem.* **1951**, 16, 1345.
- (282) Feutrill, G. I.; Mirrington, R. N. *Tetrahedron Lett.* **1970**, 16, 1327.
- (283) Draths, K. M.; Frost, J. W. *J. Am. Chem. Soc.* **1995**, 117, 2395.
- (284) Renganathan, V.; Johnston, J. B. *Appl. Microbiol. Biotechnol.* **1989**, 31, 419-424.
- (285) Yoshida, Y.; Ikura, Y.; Kudo, T. *Biosci. Biotechnol. Biochem.* **1997**, 61, 46.
- (286) Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P. *Can. J. Chem.* **1982**, 60, 1374.
- (287) Malcolm, S. A.; Sofowora, E. A. *Lloydia* **1969**, 32, 512.
- (288) Mwauluka, K.; Charlwood, B. V.; Briggs, J. M.; Bell, E. A. *Biochem. Physiol. Pflanzen* **1975**, 168, 15.
- (289) Watt, J. M.; Breyer-Brandwijk, M. G. *The Medicinal and Poisonous Plants of Southern and Eastern Africa*; E. & S. Livingstone, Ltd.: London, 1962.
- (290) Pettit, G. R.; Kamano, Y.; Aoyagi, R.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Rudloe, J. J. *Tetrahedron* **1985**, 41, 1985.
- (291) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. *J. Nat. Prod.* **1987**, 50, 119.
- (292) Hamel, E.; Lin, C. M. *Biochem. Pharmacol.* **1983**, 32, 3864.
- (293) Wilson, L.; Bamburg, J. R.; Mizel, S. B.; Grisham, L. M.; Creswell, J. M. *Fed. Proc.* **1974**, 33, 158.
- (294) Lelleher, J. K. *Mol. Pharmacol.* **1977**, 13, 232.
- (295) Schiff, P. B.; Kende, A. S.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1978**, 85, 737.
- (296) Loike, J. D.; Brewer, C. F.; Sternlicht, H.; Gensler, W. J.; Horwitz, S. B. *Cancer Res.* **1978**, 38, 2688.
- (297) Dixon, M.; Webb, E. C.; Thorne, C. J. R.; Tipton, K. F. *Enzymes*; Academic Press: New York, 1979.
- (298) Furstner, A.; Nikolakis, K. *Liebigs Ann. Chem.* **1996**, 2107.
- (299) Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Res.* **1997**, 57, 1829.

- (300) Pettit, G. R.; Temple, C.; Narayanan, V. L.; Varma, R.; Simpson, M. J.; Boyd, M. R.; Renner, G. A.; Bansal, N. *Anti-Cancer Drug Des.* **1995**, *10*, 299.
- (301) Pettit, G. R.; Rhodes, M. R. *Anti-Cancer Drug Des.* **1998**, *13*, 183.
- (302) Pettit, G. R.; Toki, B. E.; Herald, D. L.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Chapuis, J. C. *J. Med. Chem.* **1999**, *42*, 1459.
- (303) Getahun, Z.; Jurd, L.; Chu, P. S.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1992**, *35*, 1058.
- (304) Sackett, D. L. *Pharmac. Ther.* **1993**, *59*, 163.
- (305) Medarde, M.; Pelaez-Lamamie de Clairac, R.; Ramos, A. C.; Caballero, E.; Lopez, J. L.; Gravalos, D. G.; San Feliciano, A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 229.
- (306) Olszewski, J. D.; Marshalla, M.; Sabat, M.; Sundberg, R. J. *J. Org. Chem.* **1994**, *59*, 4285.
- (307) Woods, J. A.; Hadfield, J. A.; Pettit, G. R.; Fox, B. W.; McGown, A. T. *Br. J. Cancer* **1995**, *71*, 705.
- (308) Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1991**, *34*, 2579.
- (309) Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H.-M.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1992**, *35*, 2293.
- (310) Baker, W.; Savage, R. I. *J. Chem. Soc.* **1938**, 1602.
- (311) Baker, W.; Evans, C. *J. Chem. Soc.* **1938**, 372-5.
- (312) Pettit, G. R.; Singh, S. B.; Cragg, G. M. *J. Org. Chem.* **1985**, *50*, 3404.
- (313) Lawrence, N. J.; Ghani, F. A.; Hepworth, L. A.; Hadfield, J. A.; McGown, A. T.; Pritchard, R. G. *Synthesis* **1999**, *9*, 1656.
- (314) Gaukroger, K.; Hadfield, J. A.; Hepworth, L. A.; Lawrence, N. J.; McGown, A. T. *J. Org. Chem.* **2001**, *66*, 8135.
- (315) Pettit, G. R.; Singh, S. B.; Niven, M. L. *J. Am. Chem. Soc.* **1988**, *110*, 8539.
- (316) Singh, S. B.; Pettit, G. R. *J. Org. Chem.* **1990**, *55*, 2797.
- (317) Boger, D. L.; Sakya, S. M.; Yohannes, D. *J. Org. Chem.* **1991**, *56*, 4204.

- (318) Bollag, D. M.; McQuencey, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325.
- (319) Brossi, A.; Yeh, H. J. C.; Chrzanowska, M.; Wolff, J.; Hamel, E.; Lin, C. M.; Quin, F.; Stufness, M.; Silverton, J. *Med. Res. Rev.* **1988**, *8*, 77.
- (320) Couladouros, E. A.; Soufli, I. C. *Tetrahedron Lett.* **1994**, *35*, 4409.
- (321) Couladouros, E. A.; Soufli, I. C.; Moutsos, V. I.; Chadha, R. K. *Chem. Eur. J.* **1998**, *4*, 33.
- (322) Yoon, N. M.; Gyoung, Y. S. *J. Org. Chem.* **1985**, *50*, 2443.
- (323) Mitsunobu, O. *Synthesis* **1981**, 1.
- (324) Justus, K.; Steglich, W. *Tetrahedron Lett.* **1991**, *32*, 5781.
- (325) Garegg, P. J.; Samuelsson, B. *J. Chem. Soc. Chem. Commun.* **1979**, 978.
- (326) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768.
- (327) Boigegrain, R.; Castro, B. *Tetrahedron Lett.* **1976**, 1283.
- (328) Robinson, P. L.; Barry, C. N.; Bass, S. W.; Jarvis, S. E.; Evans, S. A. J. *J. Org. Chem.* **1983**, *48*, 5396.
- (329) Hudlicky, T.; Novak, B. *Tetrahedron Asymmetry* **1999**, *10*, 2067.
- (330) Bui, V.; Vidar Hansen, T.; Stenstrom, Y. R.; W., D.; Hudlicky, T. *J. Chem. Soc., Perkin Trans. I* **2000**, *11*, 1669-1672.
- (331) Bui, V. P.; Vidar Hansen, T.; Stenstrom, Y.; Hudlicky, T.; Ribbons, D. W. *New J. Chem.* **2001**, *25*, 116-124.
- (332) Bui, V. P.; Nguyen, M.; Hansen, J.; Baker, J.; Hudlicky, T. *Canadian Journal of Chemistry* **2002**, *80*, 708-713.
- (333) Gibson, D. T.; Gschwendt, B.; Yeh, W. K.; Kobal, V. M. *Biochemistry* **1973**, *8*, 1520.
- (334) Cripps, R. E.; Trudgill, P. W.; Whateley, G. *Eur. J. Biochem.* **1978**, *86*, 175.
- (335) Boyd, D. R.; Sharma, N. D.; Bowers, N. I.; Duffy, J.; Harrison, J. S.; Dalton, H. J. *Chem Soc., Perkin Trans. I* **2000**, 1345.
- (336) Ahmed, S. PhD, Imperial College of Science, 1991.

- (337) Simon, M. J.; Osslund, T. D.; Saunders, R.; Ensley, B. D.; Suggs, S.; Harcourt, A.; Suen, W. C.; Cruden, D. L.; Gibson, D. T. *Gene* **1993**, *127*, 31.
- (338) Paquette, L. A.; Kuo, L. H.; Doyon, J. *Tetrahedron* **1996**, *52*, 11625-11636.
- (339) Garcia Martinez, A.; Herrera, A.; Martinez, R.; Teso, E.; Garcia, A.; Osio, J.; Pargada, L.; Unanue, R.; Subramanian, L. R.; Hanack, M. *Journal of Heterocyclic Chemistry* **1988**, *25*, 1237-41.
- (340) Scott, W. J.; Crisp, G. T.; Stille, J. K. *Org. Synth.* **1990**, *68*, 116.
- (341) Koch, S. S. C.; Chamberlin, A. R. *J. Org. Chem.* **1993**, *58*, 2725-37.
- (342) Tamura, M.; Kochi, J. *Synthesis* **1971**, 303.
- (343) Baldwin, J. E.; Adlington, R. M.; Coates, J. B.; Crabbe, M. J.; Crouch, N. P.; Keeping, J. W.; Knight, G. C.; Schofield, C. J.; Ting, H. H.; Vallejo, C. A. *BIOCHEMICAL JOURNAL* **1987**, *245*, 831.
- (344) Palissa, H.; von Dohren, H.; Kleinkauf, H.; Ting, H. H.; Baldwin, J. E. *JOURNAL OF BACTERIOLOGY* **1989**, *171*, 5720.
- (345) Pang, C. P.; Chakravarti, B.; Adlington, R. M.; Ting, H. H.; White, R. L.; Jayatilake, G. S.; Baldwin, J. E.; Abraham, E. P. *BIOCHEMICAL JOURNAL* **1984**, *222*, 789.
- (346) Baldwin, J. E.; Adlington, R. M.; Domayne-Hayman, P. P.; Knight, G. C.; Ting, H. H. *J. Chem Soc., Chem. Commun.* **1987**, 1661.
- (347) Carredano, E.; Karlsson, A.; Kauppi, B.; Choudhury, D.; Perales, R. E.; Perales, J. V.; Lee, K.; Gibson, D. T.; Eklund, H.; Ramaswamy, S. *J. Mol. Biol.* **2000**, *296*, 701.
- (348) Birladeanu, L.; Hanafusa, T.; Johnson, B.; Winstein, S. *J. Am. Chem. Soc.* **1966**, *88*, 2316.
- (349) Poulter, C. D.; Winstein, S. *J. Am. Chem. Soc.* **1969**, *91*, 3649.
- (350) Poulter, C. D.; Winstein, S. *J. Am. Chem. Soc.* **1969**, *91*, 3650.
- (351) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
- (352) Smith, D. M.; Nicolaides, A.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1998**, *120*, 10223.

- (353) Frisch, M. J. T.; G.W., T.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98. Revision A.7. Gaussian, Inc., Pittsburgh PA. 1998.*
- (354) Hariharan, P. C.; Pople, J. A. *Theor. Chem. Acta* **1973**, *28*, 213.
- (355) Raghavachari, K.; Trucks, G. W. *J. Chem. Phys.* **1989**, *91*, 1062.
- (356) Newcomb, M.; Glenn, A. G. *J. Am. Chem. Soc.* **1989**, *111*, 275.
- (357) Tanko, J. M.; Drumright, R. E. *J. Am. Chem. Soc.* **1990**, *112*, 5362.
- (358) Tanko, J. M.; Drumright, R. E.; Suleman, N. K.; Brammer, L. E. *J. Am. Chem. Soc.* **1994**, *116*, 1785.
- (359) Tanko, J. M.; Phillips, J. P. *J. Am. Chem. Soc.* **1999**, *121*, 6078.
- (360) Beckwith, A. L. J.; Bowry, V. W. *J. Am. Chem. Soc.* **1994**, *116*, 2710.
- (361) Boyd, D. R.; Hand, M. V.; Sharma, N. D.; Chima, J.; Dalton, H.; Sheldrake, G. N. *J. Chem. Soc., Chem. Commun.* **1991**, 1630.
- (362) Yamamura, M.; Moritani, I.; Murahashi, S. I. *J. Organomet. Chem.* **1975**, *91*, C39.
- (363) Wender, P. A.; Takahashi, H.; Witulski, B. *J. Am. Chem. Soc.* **1995**, *117*, 4720.
- (364) Wender, P. A.; Nuss, J. M.; Smith, D. B.; Suarez-Sobrinio, A.; Vagberg, J.; Decosta, D.; Bordner, J. *J. Org. Chem.* **1997**, *62*, 4908.
- (365) Wender, P. A.; Dyckman, A. J. *Org. Lett.* **1999**, *1*, 2089.
- (366) Wender, P. A.; Fuji, M.; Husfeld, C. O.; Love, J. A. *Org. Lett.* **1999**, *1*, 137.
- (367) Wender, P. A.; Dyckman, A. J.; Husfeld, C. O.; Scanio, M. J. *Org. Lett.* **2000**, *2*, 1609.
- (368) Wender, P. A.; Bi, F. C.; Brodney, M. A.; Gosselin, F. *Org. Lett.* **2001**, *3*, 2105.

- (369) Wender, P. A.; Barzilay, C. M.; Dyckman, A. J. *J. Am. Chem. Soc.* **2001**, *123*, 179.
- (370) Hudlicky, T.; Reed, J. W.; Trost, B. M., Ed.; Pergamon Press: Oxford, 1991; Vol. 5, p 899.
- (371) Frey, D. A.; Duan, C.; Ghiviriga, I.; Hudlicky, T. *Collect. Czech. Chem. Commun.* **2000**, *65*, 561.
- (372) Gonzalez, D.; Martinot, T.; Hudlicky, T. *Tetrahedron Lett.* **1999**, *40*, 3077.
- (373) Akgun, H.; Hudlicky, T. *Tetrahedron Lett.* **1999**, *40*, 3081.
- (374) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209.
- (375) Muller, E. In *Houben-Weyl: Methoden der Organischen Chemie*; Bayer, O., Ed.; Georg Thieme Verlag: Stuttgart, 1976; Vol. VI/c, p 166-202.
- (376) Jamima, R.; Okazaki, J. *Chem. Ber.* **1916**, *49*, 1482.
- (377) Daukshas, V. K.; Martinkus, R. S.; Shaltite, Z. A.; Udrenaitis, E. B. *J. Appl. Chem. USSR* **1984**, *58*, 2520.
- (378) Kurtz, A. P.; Dawson, C. R. *J. Med. Chem.* **1971**, *14*, 729.
- (379) Franck, H. G.; Stadelhofer, J. W. In *Industrial Aromatic Chemistry*; Springer-Verlag: New York, 1988, p 1983.
- (380) Varagnat, J. In *Kirk-Othmer Encyclopedia of Chemical Technology*; 3 ed.; Grayson, M., Ed.; Wiley: New York, 1981; Vol. 13, p 39.
- (381) Szemant, H. H. In *Organic Building Blocks of the Chemical Industry*; Wiley: New York, 1989, p 512.
- (382) Zbiral, E.; Wesseley, F.; Lekuman, E. *Monatsh. Chem.* **1960**, *91*, 331.
- (383) Fields, D. L.; Miller, J. B.; Reynolds, D. D. *J. Org. Chem.* **1964**, *29*, 2640.
- (384) Bui, V. P.; Hansen, T. V.; Stenstrom, Y.; Hudlicky, T. *Green Chemistry* **2000**, *2*, 263-265.
- (385) Sheldon, R. A. *Chemtech* **1994**, 38.
- (386) Hudlicky, T.; Frey, D. A.; Koroniak, L.; Claeboe, C. D.; Brammer, L. E., Jr. *Green Chemistry* **1999**, *1*, 57.
- (387) Hudlicky, T.; Oppong, K.; Duan, C.; Stanton, C.; Laufersweiler, M. J.; Natchus, M. G. *Bioorg. & Med. Chem. Lett.* **2001**, *11*, 627.

- (388) Mason, H. S. *J. Am. Chem. Soc.* **1947**, *69*, 2241.
- (389) Pettit, G. R.; Grealish, M. P.; Herald, D. L.; Boyd, M. R.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **2000**, *43*, 2731.
- (390) Brown, M. L.; Rieger, J. M.; Macdonald, T. L. *Bioorg. Med. Chem. Ber.* **2000**, *8*, 1433.
- (391) Pettit, G. R.; Lippert, J. W. I.; Herald, D. L. *J. Org. Chem.* **2000**, *65*, 7438.
- (392) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, 4467.
- (393) Cassar, L. *J. Organomet. Chem.* **1975**, *93*, 253.
- (394) Sonogashira, K. In *Metal-catalyzed Cross-coupling Reactions*; Diederich, F., Stang, P. J., Eds.; Wiley-VCH: New York, 1998, p Chapter 5.
- (395) Sonogashira, K. In *Comprehensive Organic Synthesis*; B. M. Trost, Ed.; Pergamon: New York, 1991; Vol. 3, p Chapter 2.4.
- (396) Hundertmark, T.; Littke, A. F.; Buchwald, S. L.; Fu, G. C. *Org. Lett.* **2000**, *2*, 1730.
- (397) Castanet, A.; Colobert, F.; Schlama, T. *Org. Lett.* **2000**, *2*, 3559.
- (398) Suzuki, A. *J. Organomet. Chem.* **1999**, *576*, 147.
- (399) Suzuki, A. In *Metal-Catalyzed Cross Coupling Reactions*; Diederich, F., Stang, P. J., Eds.; Wiley-VCH: Weinheim, Germany, 1998, p 49–89.
- (400) Soderquist, J. A.; Matos, K.; Rune, A.; Ramos, J. *Tetrahedron Lett.* **1995**, *36*, 2401.
- (401) Furstner, A.; Seidel, G. *Tetrahedron* **1994**, *51*, 11165.
- (402) Bui, V. P.; Hudlicky, T.; Hansen, T. V.; Stenstrom, Y. *Tetrahedron Lett.* **2002**, *43*, 2839–2841.
- (403) Orsini, F.; Pelizzoni, F.; Bellini, B.; Miglierini, G. *Carbohydr. Res.* **1997**, *301*, 95.
- (404) Pettit, G. R.; Lippert, J. W.; Herald, D. L.; Hamel, E.; Pettit, R. K. *J. Nat. Prod.* **2000**, *63*, 969.
- (405) Ebdon, J. R. *New Methods Polym. Synth.* **1991**, 1–21.
- (406) Jagur-Grodzinski, J. *Journal of Polymer Science, Part A: Polymer Chemistry* **2002**, *40*, 2116–2133.

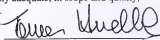
- (407) Ray, W. H. *Canadian Journal of Chemical Engineering* **1991**, 69, 626-9.
- (408) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1989**, 28, 21.
- (409) Wulff, G.; Krieger, S. *Macromol. Chem. Phys.* **1994**, 195, 3665.
- (410) Okamoto, Y.; Nakano, T. *Chem. Rev.* **1995**, 94, 349.
- (411) Crabtree, R. H. *The Organometallic Chemistry of the Transition Metals*; 3rd ed.; Wiley and Sons: New York, 2001.
- (412) Eleuterio, H. S. In *US Patent 3,074,918*, USA, 1963.
- (413) Rousch, W. R.; Hawkins, J. M.; Grubbs, R. H. *Chemtract: Org. Chem.* **1988**, 1, 21.
- (414) Okamoto, Y.; Hatada, K. *Chromatographic Chiral Separations*; Marcel Dekker: New York, 1988.
- (415) Williams, D. J. *Angew. Chem., Int. Ed. Engl.* **1984**, 23, 690.
- (416) Coates, G. W.; Waymouth, R. M. *J. Am. Chem. Soc.* **1993**, 115, 91.
- (417) Ito, Y.; Ihara, E.; Murakami, M. *Angew. Chem., Int. Ed. Engl.* **1992**, 1509.
- (418) Yokota, K.; Haba, O.; Satoh, T. *Macromol. Chem. Phys.* **1995**, 196, 2383.
- (419) Yokota, K.; Kakuchi, T.; Yamanaka, M.; Takada, Y. *Makromol. Chem., Rapid Commun.* **1987**, 7, 663.
- (420) Kakuchi, T.; Harada, Y.; Hashimoto, H.; Satoh, T.; Yokota, K. *J. Macromol. Sci., Pure Appl. Chem* **1994**, A31, 751.
- (421) Kakuchi, T.; Kawai, H.; Katoh, S.; Haba, O.; Yokota, K. *Macromolecules* **1992**, 25, 5545.
- (422) Kakuchi, T.; Haba, O.; Fukui, N.; Yokota, K. *Macromolecules* **1995**, 28, 5941.
- (423) Haba, O.; Morimoto, Y.; Uesaka, T.; Yokota, K. *Macromolecules* **1995**, 28, 6378.
- (424) Nakano, T.; Okamoto, Y.; Sogah, D. Y.; Zheng, S. *Macromolecules* **1995**, 28, 8705.
- (425) Nakano, T.; Sogah, D. Y. *J. Am. Chem. Soc.* **1995**, 117, 534.
- (426) Satoh, T.; Yokota, K.; Kakuchi, T. *Macromolecules* **1995**, 28, 4762.
- (427) Sanda, F.; Endo, T. *Macromol. Chem. Phys.* **1999**, 200, 2651.

- (428) Nakno, T. *J. Chromatogr.* **2001**, *906*, 205.
- (429) Hudlicky, T. *Chemical Reviews* **1996**, *96*, 3-30.
- (430) Paul, B. J.; Willis, J.; Martinot, T. A.; Ghiviriga, I.; Abboud, K. A.; Hudlicky, T. *Journal of the American Chemical Society* **2002**, *124*, 10416-10426.
- (431) Paul, B. J.; Martinot, T. A.; Willis, J.; Hudlicky, T. *Synthesis* **2001**, *6*, 952-956.
- (432) Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1999**, *32*, 35.
- (433) Dolhaine, H.; Hönig, H. *MATCH* **2002**, *46*, 71-89.
- (434) Dolhaine, H.; Hönig, H. *MATCH* **2002**, *46*, 91-119.
- (435) Hudlicky, T.; Abboud, K. A.; Bolonick, J.; Maurya, R.; Stanton, M. L.; Thorpe, A. J. *Chemical Communications* **1996**, *15*, 1717-1718.
- (436) Hudlicky, T.; Stabile, M. R.; Gibson, D. T.; Whited, G. M. *Organic Syntheses* **1999**, *76*, 77-85.
- (437) Hudlicky, T.; Endoma, M. A. A.; Butora, G. *J. Chem. Soc., Perkin Trans. I* **1996**, *17*, 2187-2192.
- (438) Murray, R. W.; Singh, M.; Williams, B. L.; Moncrieff, H. M. *J. Org. Chem.* **1996**, *61*, 1830.
- (439) Berti, G.; Macchia, B.; Macchia, F.; Monti, L. *J. Chem. Soc. C*, **1971**, 3371.

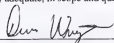
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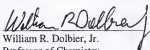
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Tomas Hudlicky, Chair  
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